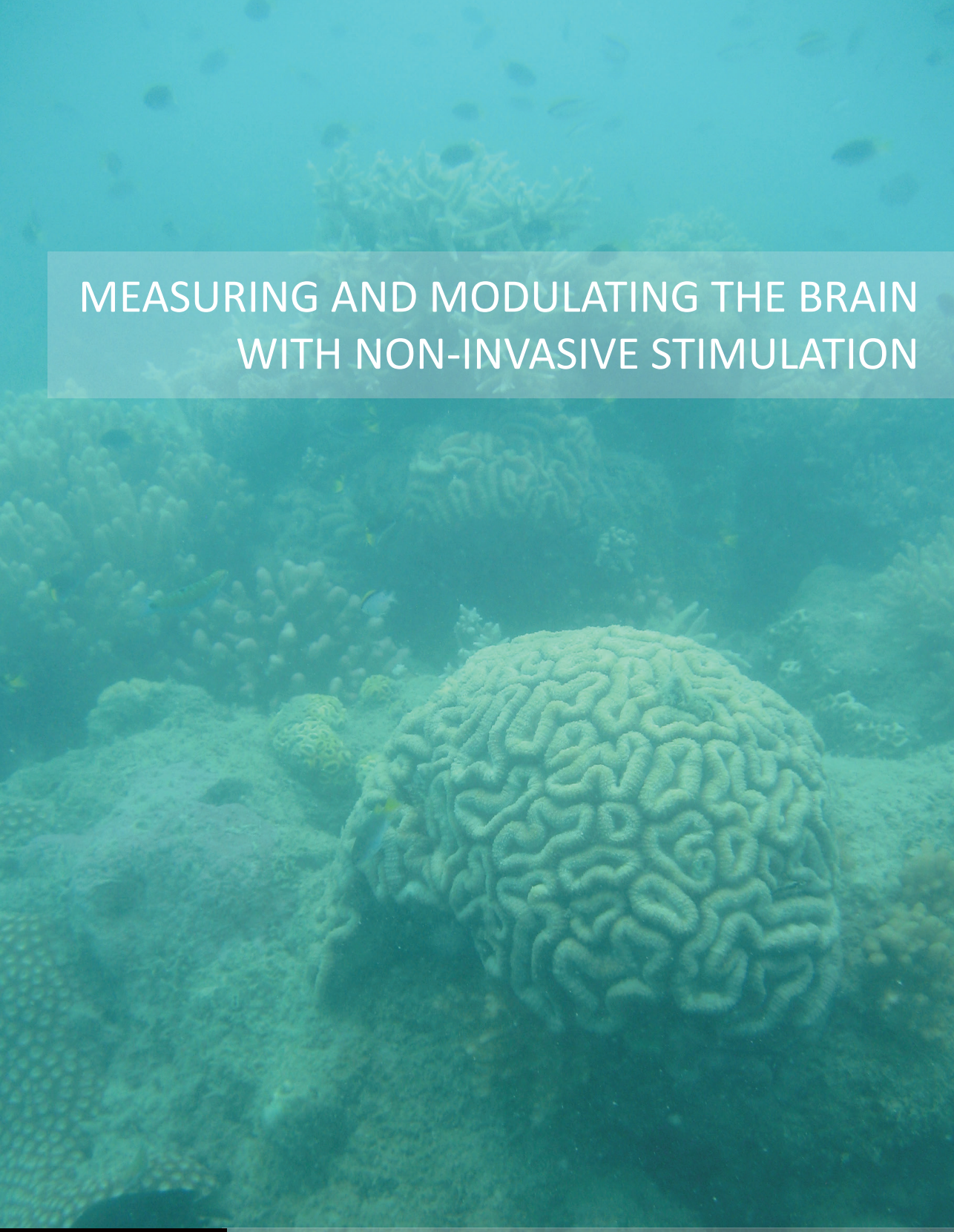
MEASURING AND MODULATING THE BRAIN WITH NON-INVASIVE STIMULATION



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MEASURING AND MODULATING THE BRAIN WITH NON-INVASIVE STIMULATION

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MEASURING AND MODULATING THE BRAIN WITH NON-INVASIVE STIMULATION

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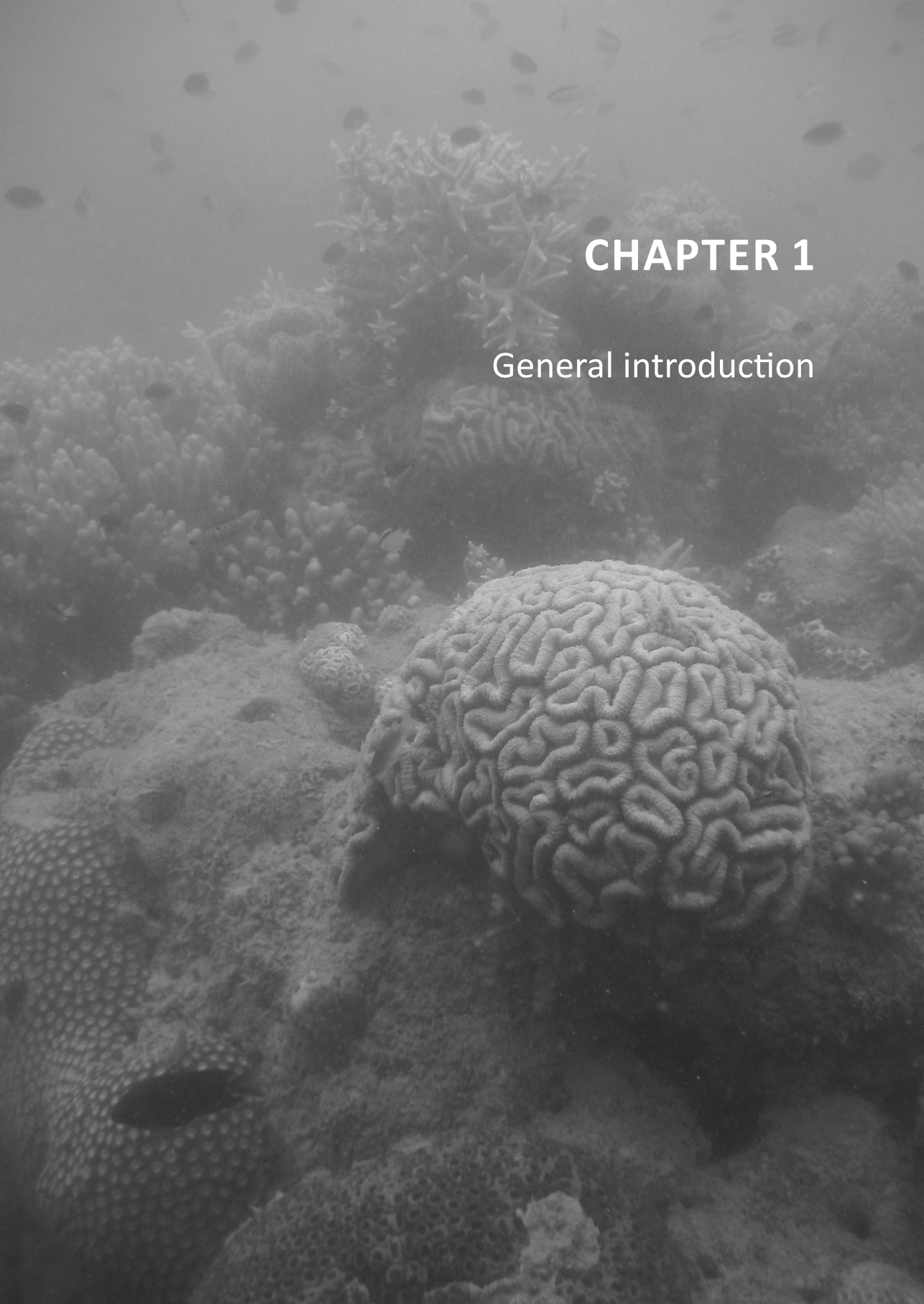

CHAPTER 1

General introduction

The human brain has been intensively investigated over the last decades. In this thesis different studies are described on measuring and modulating the brain with non-invasive stimulation. This chapter introduces principles of non-invasive brain stimulation and the outline of this thesis.

The motor system of the human brain

The brain is the most complex organ in the human body. It is the primary control center, containing billions of neurons that can simultaneously process information from inside and outside of our body, control our internal organs, generate thoughts and emotions, store and recall memories, and control movement. The main parts of the brain, responsible for the motor output, are the primary motor cortices, which communicate in an extensive cerebral network. The primary motor cortices are long stripes of cortex located in the precentral gyri, so just in front of the central sulcus (Figure 1). Via direct and indirect routes, the primary motor cortex can activate muscles or muscle groups in synergies via the corticospinal tract. The corticospinal tract descends predominantly from the cortex (upper motor neurons) through the internal capsule, midbrain, pons and medulla oblongata, where 80% of its fibers cross to the opposite (lateral) site of the spinal cord where it projects on α (lower) motor neuron pools.[1] The α motor neurons directly drive the muscles through their functional building blocks, the motor units. The most direct path from the primary motor cortex to the muscles is the main pathway for TMS induced muscle contractions (see below).

Measuring cortical excitability

History

In the 1700s and the early 1800s numerous studies of human and animal electricity are reported. Ever since the work of Galvani and Volta in the 1790s, it has been known that nerves and muscles can be stimulated with externally applied electrical currents. In electrical stimulation, charge is carried by electrons flowing in wires to the electrodes, and then transferred at the electrodes to a flow of ions in the tissue. A small fraction of the charge on these ions is transferred to nearby excitable membranes and can result in membrane depolarization. In 1831, Michel Faraday discovered the scientific principle of electromagnetic induction. Researchers investigated the effects of the electromagnetic induction on the human brain already in the later 1800's.[2]

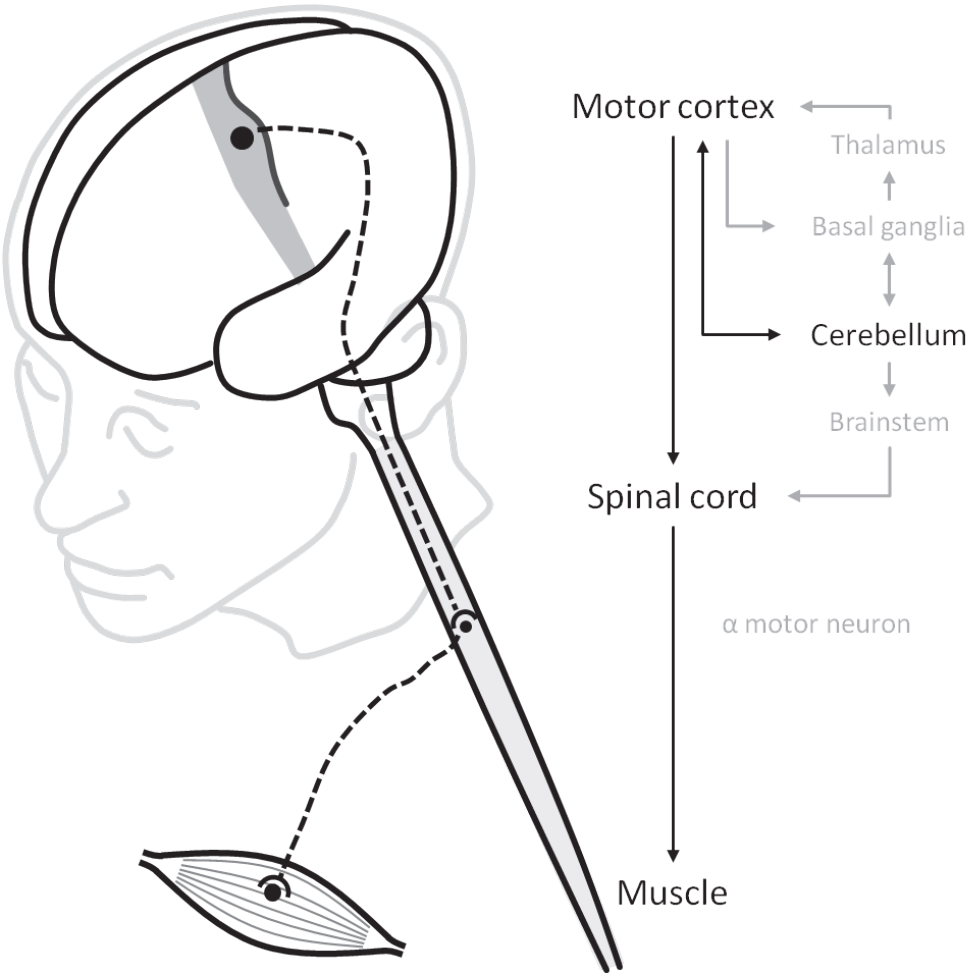

Figure 1. Schematic and simplified representation of the corticospinal system with the direct corticospinal pathway and related/connected brain structures. The primary motor cortex is a long strip of cortex located just in front of the central sulcus. The cortical neurons project to the spinal cord directly and give direct control of the α motor neurons that activate the muscles.

Transcranial magnetic stimulation

In 1985, Barker and colleagues introduced the technique of transcranial magnetic stimulation (TMS).[3] TMS works by passing a large, brief current through a copper coil placed over the scalp. By electromagnetic induction, the transient current produces a large and changing magnetic field, which induces an electric field in the underlying brain structures. Because of the conductive properties of living tissue, this field also induces a current pattern in brain structures. When a single pulse of TMS is applied over the primary motor cortex the electric field directly and/or indirectly excites the cortical motor neurons which project to the spinal

α motor neurons via the corticospinal tract. This leads to an involuntary contraction of the muscles on the opposite side of the body. The electric muscle response is called a motor evoked potential (MEP) and can be quantified using electromyography (EMG). The size of this MEP reflects the excitability of motor corticospinal output.[4] The net effect of TMS will depend on the position and orientation of the coil over a gyrus or a sulcus and the direction of the current induced. An important principle is that axons, rather than cell bodies, are preferentially activated by pulsed neurostimulation, with respect to their spatial orientation and diameter.[5] Therefore, TMS generates local activation, whereby the stimulation is at the origin of biological effects that are not only local, but also occur at a distance from the stimulation site via the activated networks.

Next to the so-called single pulse TMS, Kujirai and colleagues (1993) were the first to describe measurements of cortical inhibition and facilitation.[6] They described a paired pulse TMS technique introducing a conditioning-test paradigm. Stimulation parameters such as the intensity of the conditioning stimulus and test stimulus together with the time between the two stimuli (interstimulus interval, ISI) determine interactions between stimuli. Depending on the ISI, one can probe inhibitory (SICI) and/or facilitory (ICF) interneuronal subsystems. SICI and ICF likely have an intracortical origin. A single TMS pulse evokes multiple descending volleys in the spinal cord, termed indirect (I) waves, which are numbered according to their latencies. Further evidence that SICI is of cortical origin comes from epidural recordings showing that the I3 wave and subsequent I-waves produced by the test stimulus are suppressed.[7] ICF appears to take place in the cortex and is mediated by a neuronal population distinct from those mediating SICI.[8] The discussion on the detailed underlying mechanism is beyond the scope of this introduction.

In this thesis, single pulse and paired pulse TMS measurements were performed to assess corticospinal excitability. In the Chapters 2, 3, 4 and 6 this was done before and after the application of modulatory brain stimulation protocols as discussed in the next section.

Modulation of excitability

We investigated three brain modulation techniques (TMS, transcranial direct current stimulation [tDCS] and brain-computer interfacing [BCI]) (Figure 2). Depending on the stimulation parameters, the excitability can be decreased or increased, even beyond the duration of stimulation. Effectively, this provides an opportunity to provoke mechanisms of acute cortical reorganization in the healthy human brain [9] and in the diseased brain.

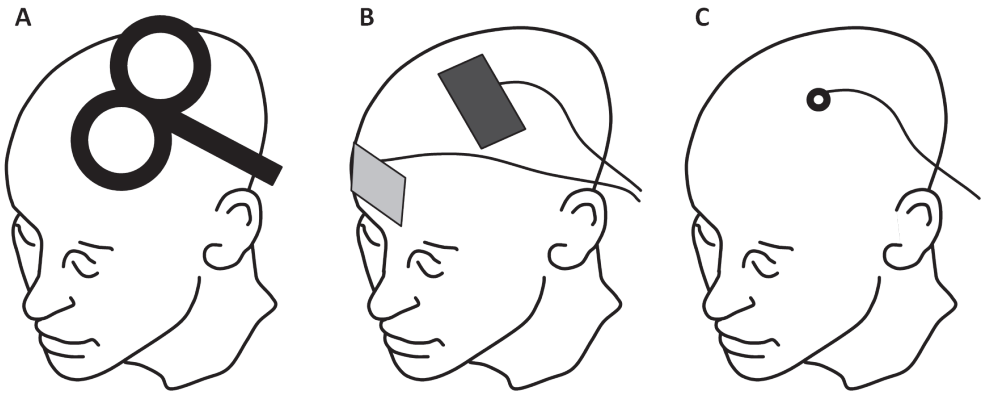

Figure 2. Schematic drawing of the application of transcranial magnetic stimulation (TMS, A), transcranial direct current stimulation (tDCS, B) and EEG recording as element in a brain-computer interface (BCI, C) over the motor cortex. The TMS coil is placed over the motor cortex to measure and/or modulate the corticospinal excitability (A). One of tDCS electrodes is placed over the motor cortex, the other above the contralateral eyebrow (B), to modulate the corticospinal excitability. For the BCI application, a EEG electrode is placed over the motor cortex location of interest and was referenced to the contralateral mastoid (not shown).

Transcranial direct current stimulation

In 1998, tDCS was reintroduced, following its use in animal experiments in the 1960's, by Priori and colleagues.[10] With tDCS, a weak constant electrical current (≥ 1 mA), which is partly passed through the skull and underlying structures to the cortical structures, up- or down- regulates cortical excitability depending on the stimulation polarity used. Cathodal tDCS over the motor cortex, whereby the cathode is placed over the primary motor cortex and the anode above the contralateral eyebrow, leads to decreased excitability of the motor cortex in healthy controls.[11] Anodal tDCS leads to increased corticospinal excitability. Cathodal tDCS is used in Chapter 2 of this thesis.

Repetitive transcranial magnetic stimulation

Besides measuring corticospinal excitability TMS can, like tDCS, also be used in modulating the excitability of the brain when applied repetitively (repetitive TMS or rTMS). Classical rTMS consists of a train of TMS stimuli with a constant frequency. Depending on that frequency the excitability is decreased (~1 Hz) or increased (5-20 Hz). The duration of stimulation determines the duration of the effect. In 2005, a form of patterned rTMS, the so called theta burst stimulation (TBS) protocol, was introduces.[12] "Theta" refers to the 5 Hz frequency by which the bursts of stimuli are applied. This TBS protocol is short (≤ 200 s) and gives longer lasting effects in healthy subjects (up to 1 hour) then the classical rTMS. With the prospect of a potential therapeutic effect, the TBS protocol was chosen in the studies described in this thesis (Chapter 3 and 4).

Brain-computer interface

Another non-invasive technique to modulate brain function is of a completely different character. We exploit brain-computer interfacing (BCI) in order to manipulate brain rhythms endogenously.[13] BCI allows real-time information of brain activity to be fed-back to a user by means of a computer in a closed "neurofeedback" loop (NFB), enabling endogenous control and natural operation of brain oscillations across cortical networks in vivo.[14]

Excitability in disease

In this thesis, non-invasive brain stimulation is discussed in connection with patients with neurological diseases. Excitability differences in disease can be measured and modulated using the above mentioned techniques. Up to now these techniques appear to be safe to use in patients. Next, a short introduction to three of the discussed diseases is given.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the upper (cortical) and lower (spinal) motor neurons. Pathologically it is characterized by motor neuron loss in the motor cortex, brainstem and spinal cord. ALS is universally fatal, with a median survival of 3 years from symptom onset.[15] ALS manifests itself clinically as progressive weakness of muscles under voluntary control.[16] The clinical features of ALS reflect the combined loss of motor neurons in the motor cortex (spasticity, increased tendon reflexes and pathological tendon reflexes) and lower motor neurons (fasciculations, muscle weakness and muscle atrophy) in the brainstem and spinal cord. Although the etiology of ALS is still unclear, hypotheses concerning disease progression have been formulated. The corticomotorneuronal (`dying-forward') hypothesis proposes that ALS is a primary disorder of the motor cortex, with lower motor neuron loss occurring secondary to excitotoxic drive from upstream sources.[17] To date, TMS studies have established motor cortical and corticospinal dysfunction in ALS [18], with cortical hyperexcitability being an early feature of sporadic ALS [19] and preceding the development of familial ALS.[20]

Parkinson's disease

Parkinson's Disease (PD) is a neurodegenerative disorder that predominantly involves the dopaminergic system of the brain. Pathologically, PD is characterized by severe loss of substantia nigra dopaminergic neurons (part of the basal ganglia). This degeneration leads to a shortage of dopamine in the striatum, especially in the putamen. This causes various movement impairments, which increase in severity during progression of the disease. The classical motor symptoms of the disease are resting tremor, bradykinesia,

rigidity and postural instability. Another invalidating motor symptom, in some of the PD patients, is freezing of gait (FOG), which is a disabling clinical phenomenon characterized by brief episodes of inability to step or by extremely short and rapid steps. It causes mobility problems and is one of the most common causes of falls in PD. The brain mechanism behind the occurrence of FOG is still not completely clear and further research is needed. Some studies indicate that connections between the frontal cortex and the basal ganglia fail and cerebellar input to the cortex compensates part of this failure.[21]

Epilepsy

Epilepsy is a disorder characterized by an enduring predisposition to generate seizures. Abnormally increased excitation and excitability of the cerebral cortex are fundamental to epilepsy.[22] Conceptually, excitability of the cortex is abnormal even in the absence of a seizure. During a seizure excitation spreads through a larger network such that dysfunction of a substantial part of the brain will cause symptoms. The diagnosis and classification of epilepsy is usually supported by an electroencephalogram (EEG), with epileptiform activity being a correlate of abnormal excitation. With TMS it is possible to measure excitability more directly. Excitability differences have been demonstrated for different epilepsy syndromes, just before and after a seizure, and after sleep deprivation. TMS measurements in healthy siblings of patients with both generalized and focal epilepsy showed also reduced intracortical inhibition.[23]

Outline of this thesis

The main theme of this thesis is measuring and modulating the corticospinal excitability and to study the possibility of therapeutic modulation of excitability in a number of neurological disorders. Chapter 2 en 3 describe the studies in which, it was studied whether the cortical hyperexcitability in ALS patients could be changed by tDCS (Chapter 2) and cTBS (Chapter 3). These pilot studies were performed to estimate the value of these techniques in diagnostic and therapeutic sense. Chapter 4 describes the study of the role of the cerebellum in PD on movement related hand/finger freezing. We investigated whether it is possible to decrease the number of freezing episodes by stimulating the cerebellum with TBS. Chapter 5 describes a review of TMS studies and the effects of antiepileptic drugs on cortical excitation and inhibition in epilepsy. Chapter 6 describes the effect of a BCI protocol on the excitability of the motor cortex in healthy subjects. This chapter describes the effect of the BCI intervention on the excitability of the motor cortex measured with TMS. Chapter 7 describes a methodological study on the positioning of EMG electrodes for TMS measurements of forearm muscles to obtain optimal en maximal MEP, applicable for instance in the prediction of hand/am recovery after stroke.

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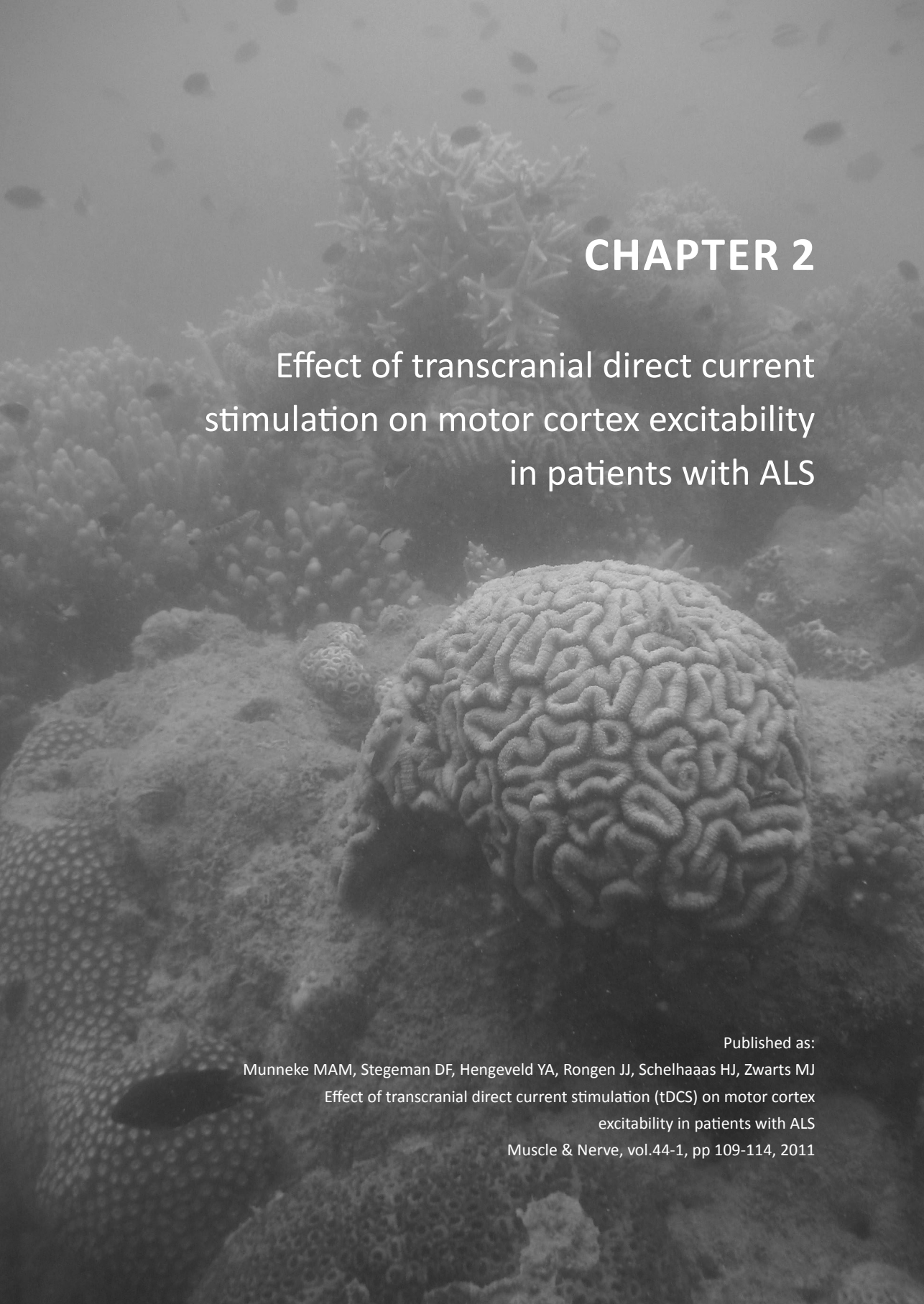

CHAPTER 2

Effect of transcranial direct current stimulation on motor cortex excitability in patients with ALS

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Effect of transcranial direct current stimulation (tDCS) on motor cortex excitability in patients with ALS
Muscle & Nerve, vol.44-1, pp 109-114, 2011

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive disease caused by the degeneration of upper and lower motor neurons. The etiology of ALS is unclear, but there is evidence that loss of cortical inhibition could be related to motor neuron degeneration. We studied whether cathodal transcranial direct current stimulation (tDCS) can reduce cortical excitability in patients with ALS. Three sessions of cathodal tDCS, lasting 7, 11 or 15 minutes were performed in 10 patients and 10 healthy controls. Corticospinal excitability was measured before and after the tDCS. Cathodal tDCS induced a consistent decrease in corticospinal excitability in healthy controls, but not in ALS patients. The failure of tDCS to produce an excitability shift in the patients supports the potential diagnostic value of tDCS as a marker of upper motor neuron involvement. However, variation in corticospinal excitability measurements both inter- and intra-individually will limit its usefulness.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by the degeneration of both the upper and lower motor neurons that control voluntary muscle movement. Although the exact etiology of ALS is unclear, loss of inhibition in motor cortex circuits has been described in patients with ALS, particularly early in the disease.[1] It is speculated that loss of inhibition not only causes central motor neuron loss but also drives anterior horn cells into metabolic deficit, a process called anterograde degeneration.[2]

A decade ago, a non-invasive tool to modulate cortical excitability, transcranial direct current stimulation (tDCS), was reintroduced.[3] With tDCS, a weak constant electrical current (≤ 1 mA), which is partly passed through the skull and underlying structures to the cortical structures, up- or downregulates cortical excitability depending on the stimulation polarity used. Cathodal tDCS over the motor cortex, where the cathode is placed over the primary motor cortex and the anode above the contralateral eyebrow, leads to decreased excitability of the motor cortex in healthy controls, evidenced by decreased muscle responses elicited by transcranial magnetic stimulation (TMS).[4-17] If tDCS is applied for several minutes, the changes can outlast the stimulation by up to 1 hour.[11, 18] Given cortical disinhibition in patients with ALS, cathodal tDCS is considered a proposed treatment option. In healthy subjects, stimulation for at least 3 minutes at 1 mA already elicits an after effect.[10] Stimulation for up to 15 minutes at 1 mA is without noticeable side effects.[19] Thus stimulation for 3-15 minutes appears to be safe and effective.

Only one study has investigated the effects of tDCS stimulation in patients with ALS.[6] Anodal and cathodal tDCS, performed for 7 minutes led to a consistent modification of cortical excitability in healthy subjects, but not in patients with ALS. However, in this study the duration of tDCS stimulation was not varied, even though studies of healthy individuals have shown that the duration of stimulation influences the extent and duration of cortical modulation.[11, 18] The authors suggested that tDCS might be useful as a diagnostic tool for ALS. They did not discuss the potential of tDCS as a therapeutic strategy. Obviously, to have a therapeutic effect on the continuous process of anterograde degeneration, cortical modulation needs to be present, but also it must be long lasting.

The first aim of our study was to address the potential of tDCS as therapeutic strategy. The second aim was to further investigate the diagnostic potential of short duration tDCS as reported by Quartarone *et al*.[6] For this, we studied the effect of lengthening the tDCS stimulation up to 15 minutes in an attempt to induce lasting changes in cortical excitability.

Methods

Subjects

Ten patients with sporadic ALS and 10 healthy controls participated in this study. All patients were categorized as having clinically probable ALS according to the revised El Escorial criteria.[20] In all patients and controls we were able to consistently elicit MEPs in the contralateral target muscle with a mean peak-to-peak amplitude of at least 1 mV. At the time of the study all patients were on Riluzole. The patients were recruited from the National ALS center. The controls were recruited through posters and flyers displayed in the Radboud University Nijmegen Medical Center.

All patients and controls gave written informed consent prior to inclusion in the study. The study was approved by the ethics committee of the Radboud University Nijmegen Medical Center and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Transcranial direct current stimulation

The study protocol consisted of three experimental sessions separated by 1 week. In each session, participants received cathodal tDCS (1 mA) for either 7, 11, or 15 minutes (in random order). We did not use anodal tDCS, because only cathodal tDCS is expected to be potentially effective in patients with ALS. tDCS was delivered using a constant-current stimulator (Eldith, neuroConn GmbH, Ilmenau, Germany) via two conductive rubber electrodes (35 cm^2) inside saline-soaked sponges placed on the scalp. The cathode was placed over the left primary motor hand area and the anode above the right eyebrow. Before the electrodes were placed, the skin was rigorously cleaned and lightly abraded to reduce impedance. The target skin impedance, as measured by the stimulator, was < 15 kΩ. To avoid abrupt sensations, the stimulation period was initiated by a fade-in period (10 s) and completed by a fade-out period (10 s).

Transcranial magnetic stimulation

We used various well-established TMS paradigms [21] to compare corticospinal excitability of the stimulated left primary motor hand area before (baseline, 0) and 5 and 20 minutes after tDCS. All TMS measurements were performed using two monophasic Magstim 200^2 magnetic stimulators (Magstim Co., Whitland, Wales, UK), which were connected through a $BiStim^2$ User Interface module to a standard circular coil (diameter 90 mm, Magstim) centered above the vertex with the A-side visible. Each stimulus induces a anticlockwise current, which resulted in posterior-anterior current flow in the left hemisphere. Because of non-focal stimulation with tDCS, we measured excitability with a round non-focal coil. Several measures of corticospinal excitability were assessed with single-pulse and paired-pulse TMS.

(i) Stimulus intensity needed to evoke a MEP of 0.5 mV amplitude ($SI_{0.5mV}$). $SI_{0.5mV}$ was defined as the lowest stimulator output intensity at which a single TMS pulse induced motor-evoked potentials (MEPs) of at least 0.5 mV peak-to-peak amplitude in the right abductor digiti minimi (ADM) muscle in at least half of 10 trials. We used this measure instead of the resting motor threshold (criterion of 50 μV), because patients often had fasciculations in their hand muscles. The presence of fasciculations rendered it impossible to distinguish between small MEPs and spontaneous fasciculations when TMS was given around threshold intensity.
(ii) Single-pulse MEPs. Before tDCS, the lowest stimulator output intensity needed to induce MEPs with a mean amplitude of approximately 1 mV (SI_{1mV}) was determined from, on average, 20 consecutive trials. This intensity was used to deliver 30 consecutive pulses at, on average, 0.25 Hz (random 4, 5 and 6 second intervals).
(iii) Paired-pulse TMS was performed in each subject to investigate short-interval intracortical inhibition and facilitation (SICI and ICF).[22] The conditioning subthreshold stimulus was set to 80% of the $SI_{0.5mV}$ and was delivered through the same magnetic coil at interstimulus intervals of 2 and 3 ms to assess SICI and 10 and 12 ms to assess ICF before a suprathreshold test stimulus. The test stimulus intensity was set to SI_{1mV} and was kept constant throughout the experiment. This procedure allows the measurement of intracortical inhibition and facilitation, which are considered to reflect the excitability of short inhibitory and facilitatory interneuronal circuits in the motor cortex.[23] A randomized protocol was run to measure SICI and ICF. It consisted of 50 stimuli given at, on average, 0.25 Hz in blocks of 10 stimuli. Forty conditioned MEPs were recorded (10 for each ISI) and 10 unconditioned MEPs.

Procedure

During each session the participants were seated in a slightly reclining chair with the elbow semiflexed and the forearm supinated, fully relaxed, and supported by a pillow on the thigh. Prior to the TMS baseline measurements, compound muscle action potentials (CMAPs) were measured in the ADM and the abductor pollicis brevis (APB) muscles of the right hand through supramaximal peripheral stimulation of the median and ulnar nerve (6 cm proximal to the active electrodes), respectively. Stimulation was done using a Digitimer constant current stimulator (model DS7A, Digitimer Ltd, Welwyn Garden City, United Kingdom).
We used visual EMG feedback to be sure of complete relaxation of the ADM muscle. No feedback was given for the other hand muscles. We chose the ADM muscle because other commonly used muscles, such as the FDI and APB, are the most atrophic in patients with ALS (split hand [24,25]), which makes it more difficult to evoke consistent MEPs in those muscles. Obtaining the excitability measures took, on average, 10 minutes, and 1 complete session took 65 minutes.

Data acquisition

Surface electromyographic (EMG) activity of the ADM muscle was recorded using self-adhesive Ag-AgCl surface electrodes (Soft-E H69P, Kendall-LTP, Chicopee, MA) using a belly-tendon montage. EMG signals were amplified (0.6 μV/bit) and band-pass filtered between 10 and 500 Hz. The EMG signals were acquired at a rate of 10 kHz (CED 1401 Laboratory Interface, Cambridge Electronic Design, Cambridge, UK) and recorded using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Digitized recordings, running from 500 ms before to 1500 ms after each TMS trigger, were stored for further analysis.

Analysis

For each block of measurements (baseline (0), 5 and 20 min after tDCS), the peak-to-peak amplitude of each MEP (in mV) was measured off-line, and the mean MEP amplitude was calculated for each stimulation condition (single-pulse MEP, SICI, and ICF) with custom-written Matlab (The MathWorks Inc., Natick, USA) scripts. To be able to compare the responses of the individuals, the baseline values of $SI_{0.5mV}$ and single-pulse MEPs were set to 100 percent, and for the follow-up measurements the relative change was calculated.

For SICI and ICF, the ratio between the conditioned MEP and the unconditioned MEP was calculated from individual data. The SICI was calculated as the mean of ISI 2 ms and 3 ms, ICF as the mean of ISI 10 ms and 12 ms. Ratios < 1 indicate inhibition, whereas ratios > 1 indicate facilitation.

Stimulus intensities and MEP amplitudes for the different excitability measures were entered separately in three-way repeated-measures analyses of variance (ANOVA) with tDCS duration (7, 11, 15 min) and time (baseline (0), 5 and 20 min after tDCS) as within-subject factor and group (patients, controls) as between-subjects factor. The Greenhouse–Geisser method was used in case of non-sphericity. If the F-value was significant, paired-sample two-tailed t-tests were used for post hoc comparisons. For all tests, $p \leq 0.05$ was considered significant. Data are given as means ± standard error of the mean, unless otherwise indicated.

Results

Subjects

The 10 healthy controls were well matched with the 10 patients for age (ALS mean 54.0 ± 3.1 years; controls mean 57.2 ± 1.6 years, $p = 0.373$) and gender (ALS 6 male, controls 7 male, chi-square test: $p = 0.639$). The mean disease duration in patients was 24.2 ± 4.2 months. The mean score on the revised ALS functional rating scale (ALS-FRS-r) [26] was 36.6 ± 1.5. None of the 20 subjects reported adverse effects during or after the experiments. The tDCS stimulation was neither painful nor unpleasant for either the healthy controls or the patients.

Maximal CMAP amplitude of the ADM was similar in the patients and controls (11.1 ± 0.8 mV and 12.8 ± 0.5 mV, respectively; p = 0.330), whereas CMAP amplitude of the APB was significantly lower in the patients than in the controls (4.7 ± 0.5 mV and 8.4 ± 0.7 mV, respectively; p = 0.035). Neither the $SI_{0.5mV}$ (p = 0.96) nor the SI_{1mV} (p = 0.86) were significantly different between patients and controls.

Stimulus intensity for evoking MEPs of 0.5mV amplitude

Using $SI_{0.5mV}$ as the dependent variable, repeated-measures ANOVA revealed an effect of time (F = 15.38, p > 0.001), but not of tDCS duration. There was also a time x group interaction (F = 6.01, p = 0.006) indicating a difference in the responsiveness to tDCS between groups. Next to this, a significant effect in the between-subject variable group was found (F = 7.265, p = 0.015). Post hoc paired t-tests demonstrated that $SI_{0.5mV}$ was increased at 5 minutes after 7 and 11 minutes of tDCS (p = 0.043 and 0.008, respectively) and at 20 minutes after 15 minutes of tDCS (p = 0.040) in healthy controls (Figure 1a). In patients, the tDCS effects on $SI_{0.5mV}$ were inconsistent. There was only an increase in $SI_{0.5mV}$ after 7 minutes of tDCS (p = 0.02) but not after 11 or 15 min of tDCS at 5 minutes after tDCS (Figure 1b).

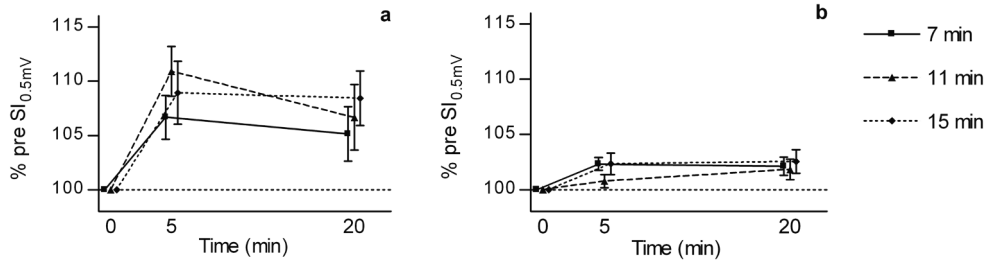

Figure 1. The $SI_{0.5mV}$ stimulus intensities given as percentage of the pre-tDCS (0 minutes) control values for healthy controls (a) and ALS patients (b) at two time-points (5 and 20 minutes) after 7 (squares), 11 (triangle), and 15 (diamond) minutes of tDCS. The error bars signify the standard error of the mean.

Single-pulse MEPs

With regard to SI_{1mV} MEP amplitude, the repeated-measures ANOVA revealed no significant effect of tDCS duration or time, and no interactions with group (Figure 2). Obviously, post hoc analysis did show a significant decrease in MEP size after 15 minutes in healthy controls (Figure 2a).

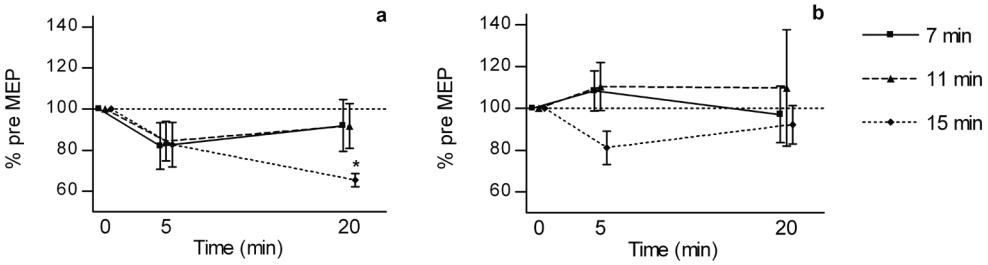

Figure 2. The single-pulse MEP amplitudes given as percentage of the pre-tDCS (0 mintues) control values for healthy controls (a) and ALS patients (b) at two time-points (5 and 20 minutes) after 7 (squares), 11 (triangle), and 15 (diamond) minutes of tDCS. The error bars signify the standard error of the mean.

Intracortical paired-pulse inhibition and facilitation

Figure 3 (SICI) and 4 (ICF) show the paired-pulse data of the healthy controls (panels 3a and 4a) and the ALS patients (panels 3b and 4b). The baseline values of SICI and ICF were similar between the groups ($p = 0.517$ and 0.107, respectively). Repeated-measures ANOVAs of the SICI revealed a time x group interaction ($F = 4.80$, $p = 0.032$), indicating that tDCS had different effects on changes in SICI over time comparing patients and controls. Although not significant for any of the tDCS durations, a reduction in SICI in healthy controls is observed, whereas the patients with ALS showed no change or only a slight change in SICI. The ANOVA of the ICF revealed a significant effect of the between-subject factor group ($F = 5.805$, $p = 0.027$). On post hoc testing, the ICF was higher overall in healthy controls compared to the ALS patients ($p < 0.01$). No effect of tDCS duration nor time nor interactions with group was found for the ICF.

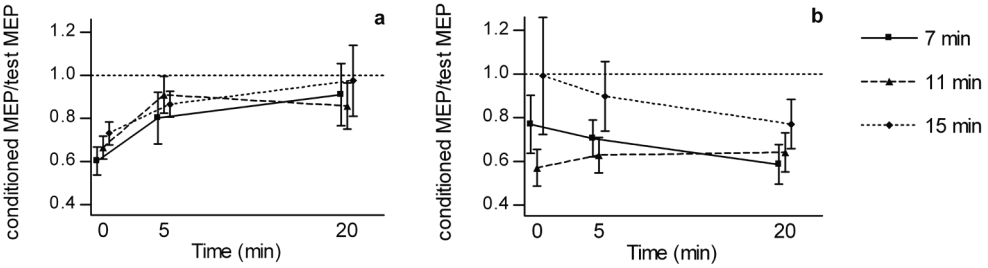

Figure 3. The short-interval intracortical inhibition (SICI) data given as ratio between the conditioned MEP and the unconditioned MEP amplitudes for healthy controls (a) and ALS patients (b) before (0 minutes) and at the two time-points (5 and 20 minutes) after 7 (squares), 11 (triangle), and 15 (diamond) minutes of tDCS. The error bars signify the standard error of the mean.

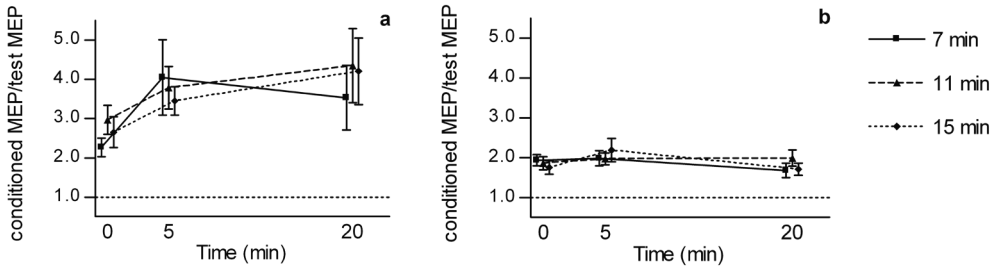

Figure 4. The intracortical facilitation (ICF) data given as ratio between the conditioned MEP and the unconditioned MEP for healthy controls (a) and ALS patients (b) before (0 minutes) and at the two time-points (5 and 20 minutes) after 7 (square), 11 (triangle), and 15 (diamond) minutes of tDCS. The error bars signify the standard error of the mean.

Discussion

Even after 15 minutes of stimulation, cathodal tDCS does not induce a decrease of cortical excitability in patients with ALS. This is in clear contrast to the results in healthy controls that do show a decrease of cortical excitability with lengthening of the stimulation duration. These results are not encouraging for a potential therapeutic effect of tDCS. However, they confirm and extend the conclusion of the only other paper that addressed the effect of tDCS in ALS. In their paper, Quartarone *et al.* extensively discussed the potential mechanisms that could underlie the lack of responsiveness in the patient group, e.g. anatomical alterations of the motor cortex and altered glutamate transmission.[6] They considered that the threshold (duration of tDCS application) for induction of the tDCS effects could be higher in ALS patients as compared to controls and that this possible explanation could have been excluded by applying longer-duration tDCS protocols. Our study, in which we doubled the stimulation duration, now indeed excludes this possibility. A ceiling effect of MEP amplitude related to the loss of cortical neurons could be another explanation, but appears unlikely, since in the study by Quartarone *et al.* the patients were at an earlier stage of disease. Also, the use of Riluzole could have influenced the abnormal tDCS respons,[27] but this is unlikely. In the Quartarone *et al.* study only one patient was on Riluzole. The lack of tDCS after-effects in patients with ALS could also be related to pathological changes in upper motor neuron membrane function.

For now, we can only speculate concerning the implications of these results for the underlying pathological upper motor neuron degeneration. Although cathodal tDCS showed decreased relative glutamate levels and gamma-aminobutyric acid in the motor cortex in healthy controls,[28] in this study we only assessed the excitability with TMS. In other studies, repetitive TMS (or theta burst stimulation) is used to change the cortical excitability in ALS.[29, 30] In these studies cortical excitability measures were not performed, and in the end a one-year of treatment did not result in reduced rate of deterioration in ALS patients.

Our study supports the suggestion of Quartarone *et al.*[6] that an abnormal tDCS effect might be a neurophysiological feature of ALS. It raises the question whether tDCS could be a diagnostic tool for ALS[32] or for the detection of early upper motor neuron involvement in ALS. However, the large variability in the TMS responses with respect to the single MEP amplitudes, SICI and ICF, as described earlier [31-33] which are not explained by age, gender or disease duration (data not shown) will limit the diagnostic potential of the protocols applied. We conclude that a single session of cathodal tDCS does not produce an excitability shift in patients with ALS. This is in contrast to the effect of tDCS in healthy controls, where tDCS can induce a decrease of cortical excitability. The variability in TMS effect that is found in patients with ALS hampers its utility as a diagnostic tool, and if diagnostic studies are considered, they should be performed strictly according to the STARD criteria.[34] Our results are not encouraging for the therapeutic effect of tDCS. However, further studies are still warranted, because, to date, only 'one-session-tDCS' has been investigated: repeated cathodal tDCS sessions may provide new insights.

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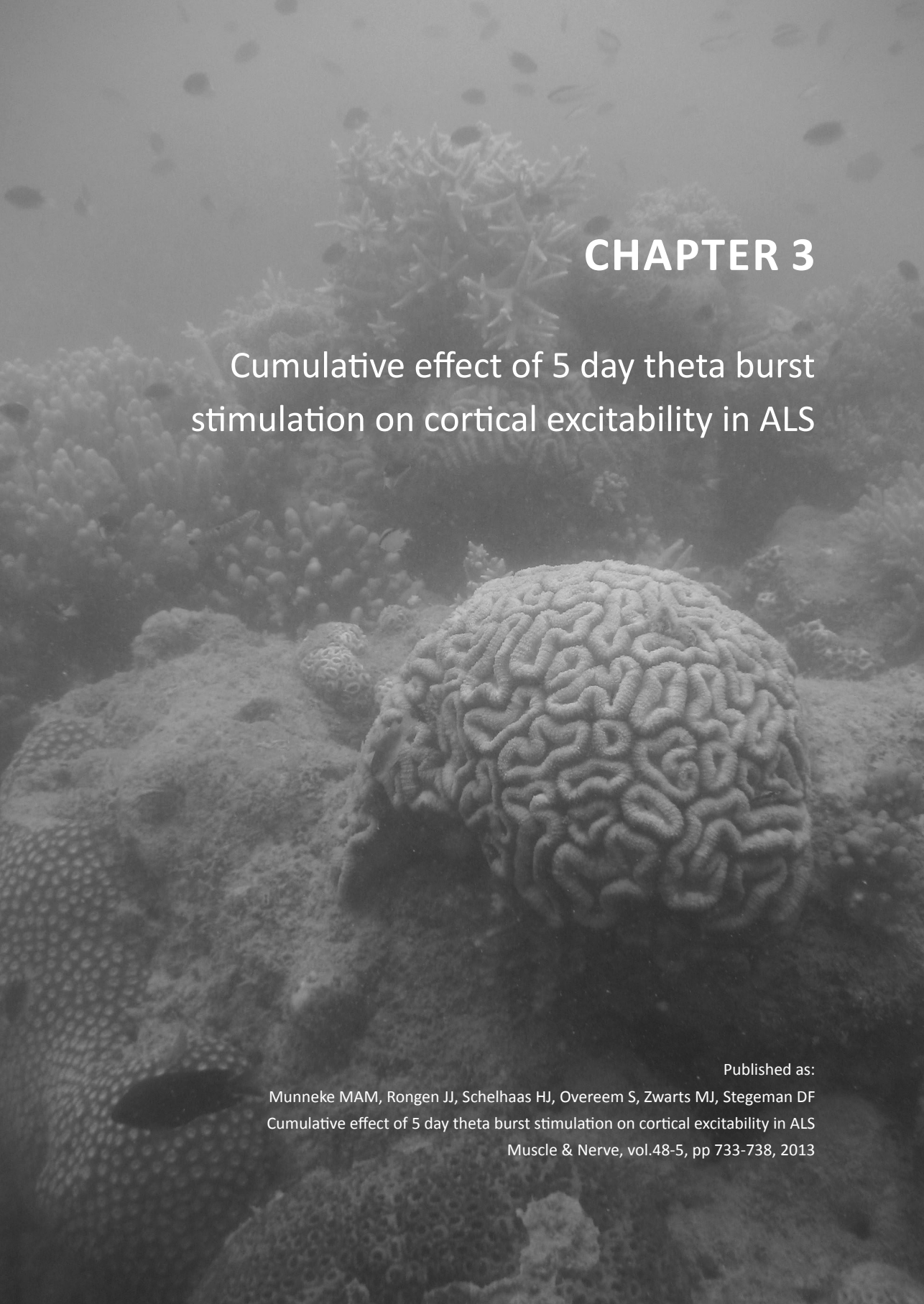

CHAPTER 3

Cumulative effect of 5 day theta burst stimulation on cortical excitability in ALS

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Abstract

Excitotoxicity plays an important role in the pathogenesis of the preferential motor neuron death observed in ALS. Continuous Theta Burst Stimulation (cTBS) by transcranial magnetic stimulation has an inhibitory effect on corticospinal excitability (CSE). We characterized the neurophysiological changes induced by cTBS in ALS. The patients received 5 daily sessions of cTBS. CSE was assessed at baseline and after each session of cTBS. The amplitude of a single pulse motor evoked potential was significantly decreased (34%) over the days. The amplitude returned to baseline a week after the last session. The resting motor threshold increased significantly, whereas intracortical inhibition and facilitation did not change over the sessions. Daily cTBS has a cumulative depressing effect on CSE in patients with ALS. Our results suggest that modulation of CSE in ALS is possible, but repetitive sessions are needed to maintain the effect.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons, resulting in the patient's death on average in 3-5 years. The pathogenesis of the preferential motor neuron degeneration in ALS has not been yet clarified. However, glutamate mediated excitotoxicity appears to play an important role.[1,2] Excitotoxicity refers to a pathological process in which increased concentrations of the excitatory neurotransmitter glutamate and related compounds destroy neurons by a prolonged increase of excitatory synaptic transmission.[3] This contributes to motor cortex hyperexcitability, which is reported to be a pathophysiological symptom in ALS.[4-6]

Transcranial magnetic stimulation (TMS) is a non-invasive method that can be used to explore the function and integrity of corticospinal pathways as well as to modulate the excitability of these systems. Such modulation can be achieved through repetitive TMS (rTMS) to apply a train of TMS stimuli.[7] Important progress was made when a new short duration protocol of conditioning the human cortex by rTMS was described with stronger and longer lasting after-effects as compared to previous protocols. This so called continuous theta burst stimulation (cTBS), applied during only 40 seconds leads to consistent, long lasting (up to 1 hour), inhibitory effects on motor cortex excitability in healthy subjects.[8]

Several rTMS studies have been performed in ALS. In contrast to the inhibiting effect of cTMS, Zanette *et al.* tested the effect of 5 Hz rTMS, administered in daily sessions over a 2-week period on motor performance, fatigue, and quality of life (QoL).[9] Results showed positive, but transitory, changes in these outcome measures and returned to normal within 2 weeks after discontinuation of rTMS. Di Lazarro *et al.* studied effects of repeated administration of cTBS. Their promising preliminary data showed a slowing of disease progression over a 6-month trial.[10] The same authors showed that cTBS of the motor cortex in patients with ALS, performed for 5 consecutive days every month for 1 year, did not have an effect on the rate of deterioration compared to patients treated with placebo stimulation.[11] To further elucidate the reasons for the above discrepancies, it is important to characterize the neurophysiological changes induced by cTBS in ALS. Therefore, the purpose of this study was to investigate corticospinal excitability in patients with ALS after cTBS. Both the effect of a single session and the longer lasting effects after repeated administration were studied. We hypothesized that the direct susceptibility of corticospinal excitability to cTBS in patients with ALS is less than in controls,[12] but that repeated administration over 5 consecutive days increases the inhibitory effects. For a feasible therapeutic opportunity such effect should also be maintained for a sufficient time after discontinuation.

Methods

Subjects

Ten right-handed men who were diagnosed with probable or definite ALS according to revised El Escorial criteria,[13] were included in the study. Inclusion criterion for patients was a spinal onset of symptoms, which were present for at least 6 and at most 36 months at time of inclusion. The mean time of onset of the symptoms was 15 (± 3) months. In addition, 10 healthy right-handed men participated. Patients as well as controls were excluded from participation if there was either a positive family history for ALS or a regular contraindication for administering TMS [a history of epilepsy or a known case of epilepsy in a first degree relative; a history of stroke or cardiac arrhythmias; a neurological disorder other than ALS; metal object(s) within the skull; or presence of a cardiac pacemaker]. All subjects completed the Edinburgh Handedness Inventory questionnaire. In addition, patients completed the revised ALS functional rating scale,[14] mean score of 43 (± 1) points.

At the time of the study all patients were on riluzole (Rilutek, Aventis Pharma BV), the standard therapy in ALS. Riluzole is the only disease-modifying therapy for ALS and partially restores hyperexcitabilty, measured as intracortical inhibition, for a short period of time.[15]

All patients and healthy subjects gave written informed consent prior to inclusion in the study. The ethics committee of the Radboud University Nijmegen Medical Center approved the study, which was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Design

Patients with ALS received 5 sessions of cTBS over 5 consecutive days (parameter TIME). Corticospinal excitability was measured at baseline (B1), after each session of cTBS (E1, E2, E3, E4, and E5), and additionally on the third and seventh day following the last session of cTBS (E8 and E12). By means of TMS, corticospinal excitability was measured before (baseline, B1) and after the application of cTBS (evaluation, E1). In order to be able to compare the effect size of cTBS on corticospinal excitability on the first and fifth day, an additional assessment of corticospinal excitability was performed before administration of the fifth session of cTBS (baseline, B5). Figure 1 shows the study design for the ALS patients. The 10 healthy age-matched subjects received a single session of cTBS. Compound motor action potentials (CMAPs) of the hand muscles were determined at B1 in both controls and patients. In patients, CMAPs were additionally determined at B5, E8, and E12. Patients were instructed to take their morning dose of Riluzole 4 hours before the scheduled appointment.

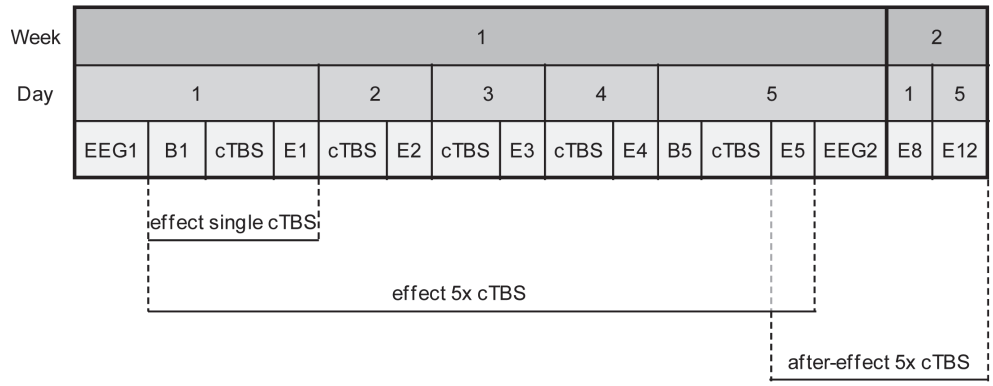

Figure 1. General study design for patients with ALS over a 2-week period. EEG = electroencephalogram, B = baseline, cTBS = continuous theta burst stimulation, E = evaluation.

Electroencephalography

All patients underwent 2 routine clinical electroencephalographic (EEG) recordings: the first before the start of the experiment (before B1) and the second after the fifth session (after E5). These recordings were made to exclude the induction of abnormal brain activity, in particular with respect to epileptiform discharges. EEGs were reviewed by an independent clinical neurophysiologist.

Electromyography

Electromyographic (EMG) activity was recorded from the right abductor polis brevis (APB) and adductor digiti minimi (ADM) muscles using self-adhesive Ag-AgCl surface electrodes (Kendall Soft-E, H59P; 35x22mm). Hand muscles were chosen, because in ALS corticospinal excitability is increased in the hand motor area at an early stage of the disease.[16] After standard skin preparation, the active electrodes were placed over the muscle in a belly-tendon montage. In order to ensure identical electrode placement over the repeated visits, we marked the location of the active electrodes with a permanent marker. Raw EMG signals were amplified (0.6 μV/bit) and bandpass filtered between 2 Hz and 2 kHz. EMG signals were digitized at 5 ksamples/s by an A/D converter (model 1401 plus, Cambridge Electronics Design, Cambridge, UK) and recorded using Spike2 software (Cambridge Electronic Design, UK). Digitized recordings, running from 500 ms before to 1500 ms after each TMS trigger, were stored for further analysis.

Compound Motor Action Potentials

CMAPs were determined in the ABP and the ADM muscles on the right hand through supramaximal peripheral stimulation of the median and ulnar nerve, respectively (6 cm proximally from the active electrodes). Stimulation was performed using a constant current stimulator (model DS7A, Digitimer Ltd, Welwyn Garden City, United Kingdom).

TMS: Continuous theta burst stimulation

TMS was delivered through a 70 mm diameter figure-of-eight coil (Magstim Company Ltd., Whitland, Wales) connected to a Magstim Super Rapid stimulator over the cortical motor area of the ABP (hotspot, see below). The basic element of cTBS is a burst of 3 stimuli at 50 Hz that is repeated every 200 ms. This pattern was repeated continuously for a period of 40 seconds (600 pulses).[8] cTBS was applied at 70% of the resting motor threshold (RMT, see next section) as determined at baseline. One feature of the inhibitory after-effects is that they take 5-10 min to reach a maximum after the end of cTBS. Corticospinal excitability was therefore examined 7 minutes after the application.[17] During the whole experiment, subjects were instructed to relax their arm and hand.

TMS: Corticospinal excitability

Single and paired pulse TMS was delivered through the same figure-of-eight coil as used for cTBS, now connected to 2 Magstim 200[2] machines connected through a BiStim[2] user interface module (Magstim Company Ltd., Whitland, Wales). The coil was held tangentially on the left hemiscalp with its handle pointing backwards at an angle of about 45 degrees from the mid-sagittal axis. Single pulse stimuli were delivered with randomized interstimulus intervals of 4, 5, or 6 seconds. All stimuli delivered through TMS were timed by trigger pulses controlled by Spike2 (Cambridge Electronic Design, UK) written software.

The hotspot, the optimal site of the magnetic coil for eliciting motor evoked potentials (MEPs) in the resting APB, was tracked at baseline. Subjects were wearing a lycra swim cap on which the location of the APB hotspot and the coil orientation were marked so that coil position could be monitored constantly. To ensure anatomically identical coil positioning over the repeated visits in patients, the location and orientation of the coil over the hotspot were saved as a target position using a stereotactic image guidance system (Brainsight, Rogue Research, Montreal, Canada).

Corticospinal excitability was assessed using a number of validated TMS techniques.[7,18] First, the resting motor threshold (RMT) was determined, defined as the minimum stimulator intensity required to obtain MEPs with an amplitude of at least 50 µV in at least 5 out of 10 trails in the relaxed right APB. Next, the minimum stimulator intensity was determined to obtain single pulse MEPs of on average 0.5 mV in patients and 1 mV in healthy controls over 10 trials, in the relaxed right APB (referred to as the reference stimulus intensity; SI_{ref}). The lower target MEP amplitude (0.5mV) in patients was adjusted for the expected disease-related reduction in CMAP amplitudes. Subsequently, the SI_{ref} was used to obtain 20 single pulse MEPs at each session. Finally, short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were assessed using the paired pulse protocol as described by Kuijrai *et al.* [19]. We used a conditioning stimulus intensity of 70% RMT and a test stimulus of SI_{ref} (each session adjusted to the current intensities). Inter-stimulus intervals of 2 and 3 ms

were used to assess SICI along with 10 and 12 ms to assess ICF. Per interstimulus interval 10 stimuli were applied, along with 10 unconditioned stimuli. A randomized protocol was used to apply the total of 50 stimuli.

Data processing

Raw EMG data from Spike2 were analyzed off line using MATLAB software (Mathworks, Natick, USA). The primary outcome measure was the amplitude of the single pulse MEP obtained with the fixed SI_{ref}. The MEP amplitude was defined as the difference between the maximum and minimum value of the raw EMG response obtained from 5 ms to 45 ms after the trigger pulse. MEPs were discarded online if the APB was not relaxed during the 200 ms preceding the actual TMS trigger (defined as a peak-peak amplitude in the voluntary EMG signal higher than 50 μV during the pre stimulus interval). Amplitudes of single pulse MEPs, were averaged per session and subsequently presented as a fraction of the average single pulse MEP amplitude obtained at baseline. Per session, MEP amplitudes obtained with the paired pulse protocol were averaged per inter-stimulus interval and presented as a fraction of the mean unconditioned test MEP amplitude. The SICI was calculated as the mean MEP after ISI 2 ms and 3 ms, ICF as the mean MEP after ISI 10 ms and 12 ms.

Statistical analysis

Demographics of both groups were tested for differences with an independent *t*-test. In healthy controls, we used a paired *t*-test to test the effects of cTBS on the different parameters (B1 and E1). In patients, we first tested for changes in corticospinal excitability and trends over the course of 5 days of cTBS. The relative amplitudes of the single pulse MEPs, relative RMT, SI_{ref}, SICI, and ICF entered the one-way repeated-measures analyses of variance (rmANOVA) separately with main factor TIME (B1, E1, E2, E3, E4, and E5). Second, we quantified for changes in corticospinal excitability and trends during follow up, using the main factor TIME (E1, E8, and E12). Third, we tested whether the effect size of cTBS on corticospinal excitability had changed over the course of repeated administration. To this end we used a paired *t*-test to compare the effect sizes of cTBS applied on day 1 (B1 - E1) with day 5 (B5 - E5). The Greenhouse-Geisser method was used in case of non-sphericity. If the F-value was significant, paired-sample 2-tailed *t*-tests were used for *post hoc* comparisons. For all tests, $P < 0.05$ was considered significant. For *post hoc* analysis a Bonferroni correction was applied [$P < (0.05/n$ comparisons)]. Data are shown as means ± standard error of the mean.

Results

All 10 patients and the 10 age-matched healthy controls completed the study. None of the subjects reported adverse effects during or after the experiments. The cTBS stimulation was neither painful nor unpleasant for either the patients or the controls. The EEG recordings showed that cTBS, repeated over 5 consecutive days, did not provoke any epileptiform discharges nor other adverse events. CMAPs did not change over the study period.
Clinical and demographic characteristics are listed in Table 1, showing differences in CMAP amplitudes, ICF, and RMT between patients with ALS and healthy controls. The whole assessment of corticospinal excitability was completed within 25 ± 1 minutes after cTBS, including the 7 minutes of rest.

Tabel 1. Demographics of the patients and controls.

	Controls	Patients	*P*-value
N	10	10	
Age (years)	49.0 ± 3.6	57.8 ± 1.8	0.08
Height (cm)	183.5 ± 2.3	177.0 ± 2.5	0.93
Weight (kg)	80.2 ± 3.7	82.0 ± 5.2	0.46
CMAP APB (mV)	13.2 ± 1.1	7.5 ± 1.2	0.01
CMAP ADM (mV)	15.8 ± 1.1	11.3 ± 1.2	0.01
SICI	54.1 ± 7.7	78.0 ± 14.0	0.11
ICF	97.9 ± 5.3	127.4 ± 15.0	0.02
RMT	40.1 ± 4.7	48.9 ± 9.9	0.04

CMAP = compound motor action potential; APB = adductor polis brevis muscle; ADM = adductor digiti minimi muscle; SICI = short-latency intracortical inhibition; ICF = intracortical facilitation; RMT = resting motor threshold.

Single sessions of cTBS in healthy controls

The single pulse MEP amplitude obtained with SI_{ref}, was lower after cTBS (-22.16 ± 8.3%; $P = 0.026$). There was no effect of cTBS on stimulator intensities, neither for RMT nor for SI_{ref} ($P = 0.148$ and $P = 0.069$, respectively). SICI did not change, but ICF was higher after cTBS (+27.9 ± 8.8%; $P = 0.011$).

Repeated sessions cTBS in patients with ALS

Single pulse MEP amplitude. One session of cTBS did not change the MEP amplitude (6%, $P = 0.653$). After the fifth session of cTBS, the MEP amplitude was significantly lower compared to baseline (34%, $P = 0.007$, Figure 2). The rmANOVA for the factor TIME showed a trend of which the linear component was significant ($F = 12.049$; *P*-value = 0.007). During follow up, the MEP amplitude returned to baseline, showing no significant difference with E1 ($F = 1.286$; $P = 0.310$). The effect size of a single cTBS session on the single pulse MEP amplitude did not differ between day 1 (B1 - E1) and day 5 (B5 - E5, $P = 0.771$).

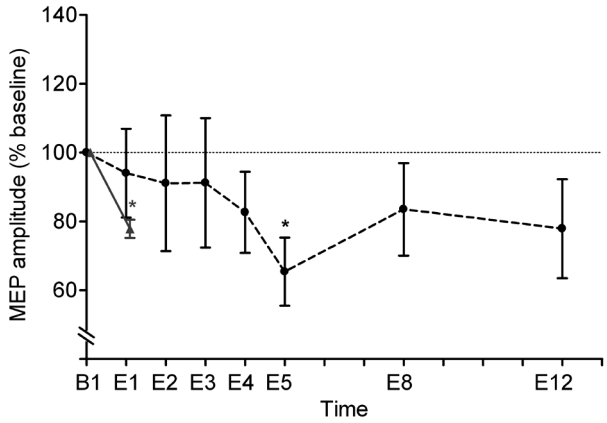

Figure 2. Relative amplitude of single pulse MEPs obtained with a fixed stimulator intensity, given as percentage of baseline (B1) values over the different evaluation sessions. In black the data of the patients, in grey the data of the healthy controls. The error bars signify the standard error of the mean. The asterisk indicates a significant difference with B1.

Stimulator thresholds. Over 5 consecutive days of cTBS, the RMT increased significantly (F = 2.522, *P* = 0.043) in a linear way (F = 7.920; *P* = 0.020). Moreover, *post hoc* analysis revealed that the RMT was significantly larger at E5 compared to B1 (*P* = 0.002, Figure 3). During follow up, the RMT decreased to baseline (F = 0.808; *P* = 0.461).

The SI_{ref} was not significantly influenced by cTBS (F = 1.818; *P* =0.129). Also in the follow up period no changes were observed.

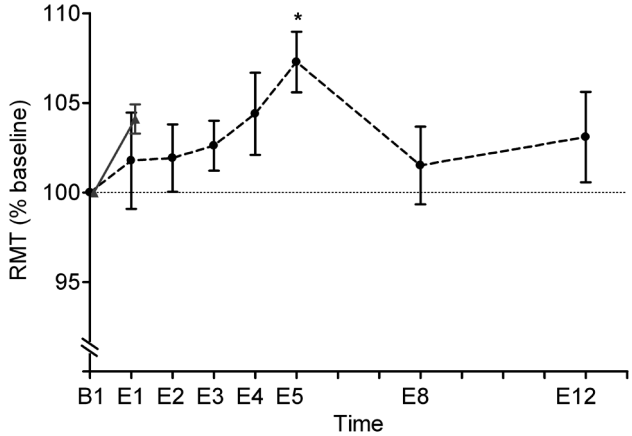

Figure 3. Relative resting motor threshold (RMT) stimulator intensity, given as percentage of baseline (B1) values over the different evaluation sessions. In black the data of the patients, in grey the data of the healthy controls. The error bars signify the standard error of the mean. The asterisk indicates a significant difference with B1.

Intracortical inhibition and facilitation. Both SICI (Supplementary figure 1) and ICF (Supplementary figure 2) were not affected by the repeated cTBS sessions [F = 0.792; *P* = 0.561 and F = 1.636; *P* = 0.225 (Greenhouse-Geisser estimate), respectively]. During follow up, SICI and ICF were not different from baseline.

Discussion

cTBS in healthy controls

As described previously, we demonstrated a decrease in corticospinal excitability after application of cTBS in healthy controls.[20] In contrast to Huang *et al.*, we observed that ICF increased after the application of cTBS, which is not in line with the assumed inhibitory properties of cTBS. Since there was a possibility of a change in RMT and SI_{ref}, we tested, other than *Huang et al.*, those variables also after cTBS and used 70% of the actual RMT and SI_{ref} at E1 for the paired pulse measures.

cTBS in patients with ALS

Although we showed that a single session of cTBS inhibits corticospinal excitability in healthy subjects, this was not the case in the patients. This may be due to disease pathology, medication use, or both. However, repeated stimulation over 5 days did modulate the excitability in ALS patients. This cumulative effect may be explained by at least 2 different mechanisms. First, it is possible that the effects through cTBS accumulate during repeated application over consecutive days. This would imply that cTBS has a constant effect on corticospinal excitability and that this effect persists in part over a minimum period of 24 hours. The effect of cTBS involves short-term changes at the level of synaptic transmission. To have an enduring and accumulating effect, it is mandatory that the short-term changes partly persist. This persistence would require new gene expression and structural changes in synaptic morphology.[21] A second explanation for the observed linear decrease is an increasing effect size of each additional cTBS session on corticospinal excitability. This explanation is based on the concept of metaplasticity. Metaplasticity refers to a higher-order form of synaptic plasticity where prior synaptic activity leads to a persistent change in the direction or magnitude of subsequent activity-dependent plasticity, without affecting actual synaptic efficacy. This implicates that cTBS may have a direct effect on both synaptic efficacy and on the sensitivity to cTBS-related changes. Changes in NMDA receptor related signalling have been proposed to cause metaplasticity.[22,23] There is also some evidence that NMDA receptors are involved in cortical modulation by TBS.[24] Hence, one can imagine that metaplasticity is involved in the after-effects of cTBS. However the similar relative effect sizes of cTBS on day 1 (B1 - E1) and day 5 (B5 - E5) give more support to the first postulated mechanism.

In addition to exploring the effect of cTBS repeated over 5 consecutive days, we also tracked corticospinal excitability on days 8 and 12 in the week after the cTBS sessions. Both the single pulse MEP amplitude and the RMT returned to baseline (B1) during this period. This suggests that cTBS repeated over 5 consecutive days induced transitory effects that did not last until the end of the second week. No changes in the intracortical excitability (SICI and ICF) were induced by the cTBS sessions. These results could explain the lack of effects on disease progression in the 1-year trial of Di Lazzaro *et al.* [11] using 3-week intervals between 5 consecutive day sessions with cTBS. A more frequent application with cTBS seems to be necessary to sustain the lower excitability of the motor cortex for longer periods.

Study limitation

The ALS patients were all using riluzole during this study, which is known to modulate excitatory neurotransmission. Stefan *et al*. investigated the long-term effect of riluzole use on cortical excitability and found that only the SICI was increased by the drug; the MEP amplitude was not affected.[15] Since riluzole has several mechanisms of action [25] we cannot be sure of its contribution to our cTBS effects. Further research should be performed to investigate the effect of riluzole on the after effects of cTBS.

Conclusion

We demonstrated that cTBS could be applied safely over 5 consecutive days in ALS patients. Our results suggest that it is possible to decrease corticospinal excitability in ALS patients, by means of repeating cTBS over consecutive days. We also provided evidence that an interval of 3 weeks between the cTBS sessions is too long for the effects to last. However, modulation of corticospinal excitability is possible, and future studies should investigate whether it is possible to obtain a positive effect on disease progression through more continuous forms of cortical modulation.

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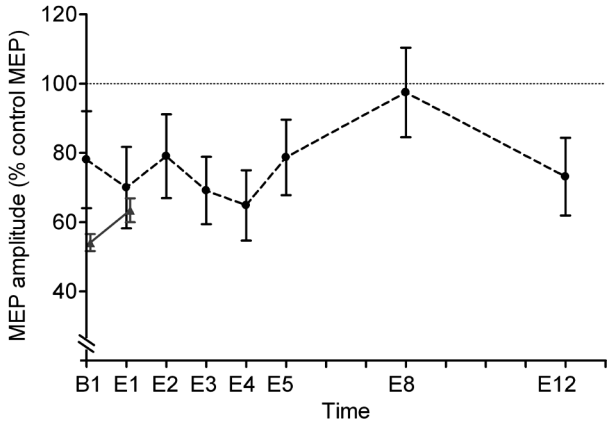
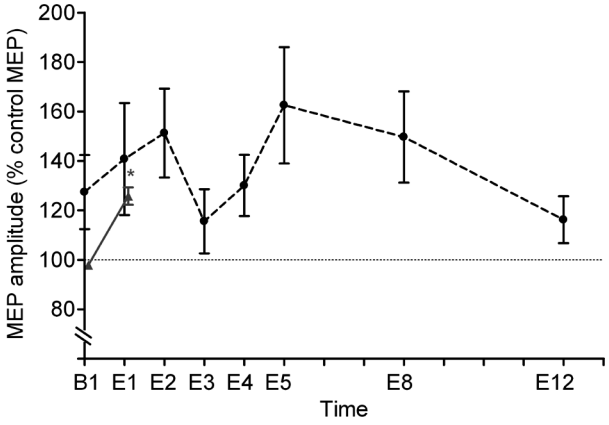
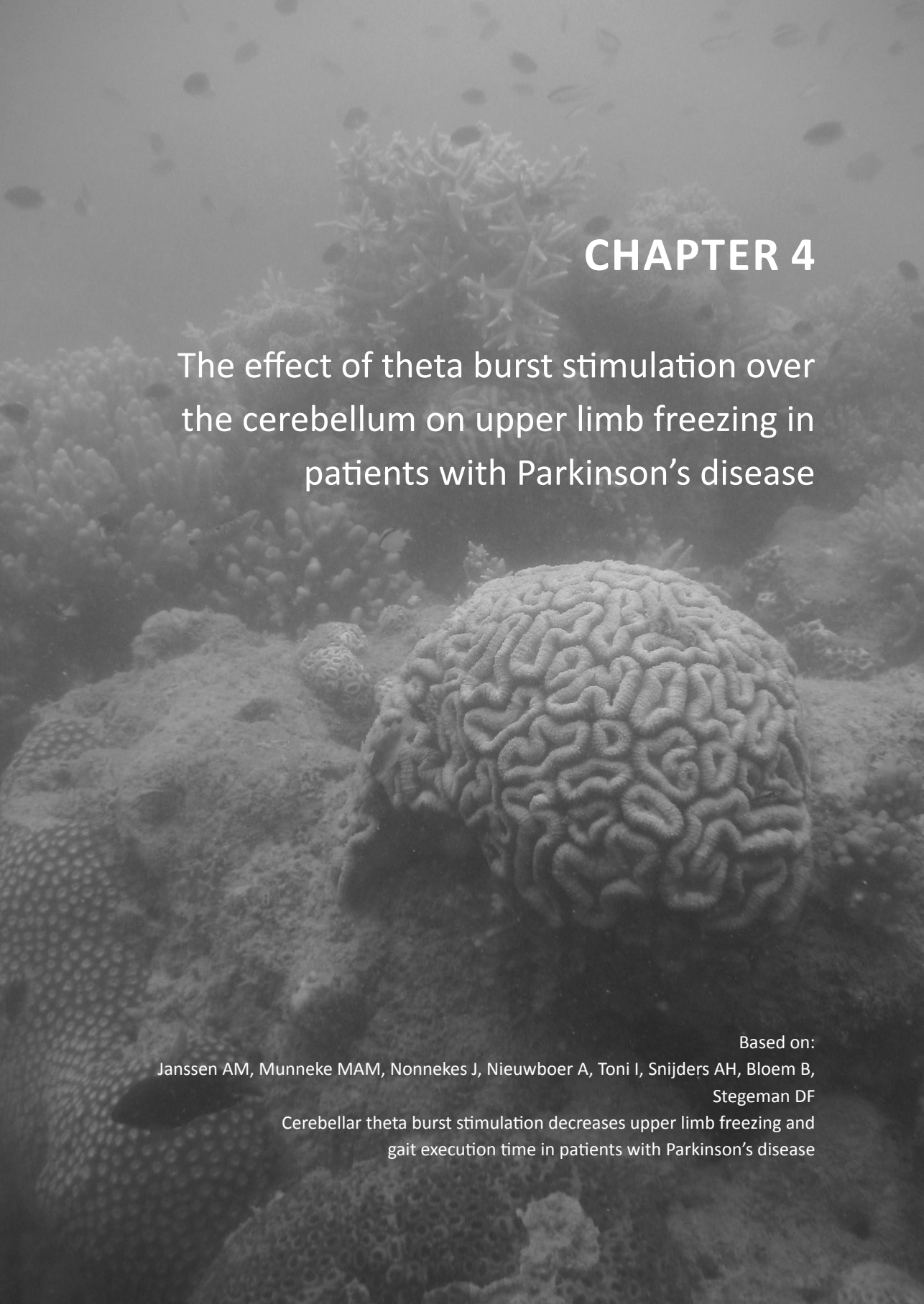


Supplementary figure 1. The short-interval intracortical inhibition (SICI) data given as the ratio between the conditioned MEP and the unconditioned MEP amplitudes over the different evaluation sessions. In black the data of the patients, in grey the data of the healthy controls. The error bars signify the standard error of the mean.



Supplementary figure 2. The intracortical facilitation (ICF) data given as the ratio between the conditioned MEP and the unconditioned MEP amplitudes over the different evaluation sessions. In black the data of the patients, in grey the data of the healthy controls. The error bars signify the standard error of the mean.



CHAPTER 4

The effect of theta burst stimulation over the cerebellum on upper limb freezing in patients with Parkinson's disease

Based on:

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Cerebellar theta burst stimulation decreases upper limb freezing and gait execution time in patients with Parkinson's disease

Abstract

One feature in part of the PD patients is freezing of gait (FOG), which is a unique and disabling clinical phenomenon characterized by brief episodes of inability to step or by extremely short and rapid steps. Freezing is not a phenomenon that is restricted to the lower limbs, but can also occur in the upper limbs. We hypothesize that patients with FOG are less able to recruit the cerebellum to compensate for an insufficient basal ganglia function. In this study, we want to investigate whether we are able to decrease freezing by stimulating the cerebellum with theta burst stimulation (TBS) protocols. In two separate sessions the patients receives either inhibitory (cTBS) or excitatory TBS (iTBS). A bimanual rhythmic upper limb task was performed before and after the TBS to measure freezing in the upper limbs. The *a priori* possible mechanism of cTBS inhibiting the inhibitory Purkinje cells and so increasing the cerebellar output could not be confirmed. The cTBS protocol did not have an effect on upper limb freezing. Instead, iTBS significantly decreased the total freezing duration during the upper limb task in the most affected hand.

Introduction

Parkinson's Disease (PD) is a degenerative disorder of the central nervous system, which is pathologically characterized by severe loss of substantia nigra dopaminergic neurons. The degeneration leads to a shortage of dopamine in the striatum, especially in the putamen. This causes various movement impairments, which can increase in severity during progression of the disease. One feature is freezing of gait (FOG),[1] which is a unique and disabling clinical phenomenon characterized by brief episodes of inability to step or by extremely short and rapid steps. It causes mobility problems and is one of the most common causes of falls in PD.[2]

Freezing is a phenomenon that is not restricted to the lower limbs, but can also occur in the upper limbs.[3,4] Freezing episodes in the upper limbs (FOUL) are usually preceded by a strong decrease of movement amplitude and hastened movements, which is similar to FOG in the lower limbs. Motor blocks have been reported to occur in alternating repetitive movements of the fingers. Nieuwboer *et al.*[4] found that FOULs occurred in known freezers of gait and also in a patient characterized as non-freezer. The occurrence of FOULs was correlated with FOG scores, but not with disease severity. These previous reports support the hypothesis that a generic motor control problem underlies freezing. In this study freezing in the upper limbs will be studied.

Although the clinical picture and the provoking factors of FOG are becoming more defined, the neural mechanism behind its occurrence is still not clear. A previous study showed that patients with FOG had an increased activity in the mesencephalic locomotor region in the brainstem and a decreased supplementary motor area (SMA) activity.[5] In addition, there may also be a role for the cerebello-cortical circuitry, although this has never been investigated extensively. In PD patients without FOG, the putamen, SMA and pre-SMA have been found to be hypoactive and, the cerebellum and the contralateral motor cortex hyperactive during a hand task.[6] It has been hypothesized that this hyperactivation in the ipsilateral cerebellum is a compensatory mechanism for the defective basal ganglia.[6-9]

We hypothesize that patients with FOG are less able to recruit cerebellar processes to compensate for the defective basal ganglia. In this study, we want to investigate whether we are able to decrease the number of freezing episodes by upregulating the cerebellar function with transcranial magnetic stimulation (TMS). TMS is a non-invasive method that can be used to explore the function and integrity of corticospinal pathways as well as to modulate the excitability of these systems. Modulation can be achieved through repetitive TMS (rTMS) to apply a train of TMS stimuli.[10] Important progress was made when a new short duration protocol of conditioning the human cortex by rTMS was described with stronger and longer lasting after-effects as compared to previous protocols. This so-called theta burst stimulation (TBS), applied during only 40-190 seconds leads to consistent, long

lasting (up to 1 hour), inhibitory (continuous TBS, cTBS) or excitatory (intermittent TBS, iTBS) effects on motor cortex excitability in healthy subjects.[11]

Not much research is conducted on the use of rTMS over the cerebellum in PD patients. There are several studies performed in healthy subjects with the standard TMS protocols over the cerebellum [12-15] and some with the TBS protocols.[16-18] These studies found a reduction in the cerebello-cortical connectivity after inhibitory rTMS and some found an increase after excitatory rTMS on the cerebellum. Until now there is only one study published on the effects of TBS over the cerebellum in PD patients.[19] This study reported an improvement (decrease) in the global abnormal involuntary movement score after a single and after multiple sessions of cTBS over the cerebellum in PD patients with levodopa-induced dyskinesia (LID). In our study, we want to excite the cerebellum to boost the hypothesized compensatory cerebello-cortical pathway. Following the rationale of this previous study, this would be an argument in favor of using iTBS to stimulate the cerebello-cortical pathway. On the other hand, cerebellar rTMS is expected to stimulate the Purkinje cells in the upper layer of the cerebellum.[20] These cells have an inhibitory projection onto the deep cerebellar nuclei. This deep cerebellar region has a connection through the thalamus to the motor cortex. When we want to excite the connections from the cerebellum to the cortex we might have to decrease the inhibition from the Purkinje cells. This reasoning would support the hypothesis that the inhibitory form (cTBS) instead of iTBS over the cerebellum increases the cerebellar activity in the circuit.

To test if any and which of these two opposite hypotheses holds, we stimulated the cerebellum of PD patients with FOG with both the inhibitory as well as the excitatory form of TBS.

Methods

Subjects

In total 20 patients with idiopathic Parkinson's disease were included. The subjects were selected based on having moderate disease severity (Hoehn and Yahr stage 2-3) and objectified FOG by an expert rater of FOG. Exclusion criteria were other neurological disorders than PD, presence of a deep brain stimulator, a mini mental state examination (MMSE)[21] score under 24, and defined exclusion criteria for TMS experiments.[22] All subjects gave written informed consent prior to inclusion in the study. The ethics committee of the Radboud university medical center approved the study, which was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Experimental design

Testing occurred while patients were off medication; i.e. after withholding anti-parkinsonian medications for at least 12 hours. Prior to testing, clinical data were collected including the new freezing of gait questionnaire (NFOGQ),[23] MMSE,[21] frontal assessment battery (FAB) [24] and the unified Parkinson's disease rating scale (UPDRS) part 3.[25] Subjects were stimulated with the two different TBS protocols (iTBS, cTBS) in separate sessions, at least one week apart. In each session they were stimulated with either cTBS or iTBS. In the second session they received the other TBS protocol. Cortical excitability has been tested directly before and directly after the TBS with single pulse TMS. Before and after the "single pulse TMS - (i/c)TBS - single pulse TMS" sequence, subjects had to perform a rhythmic upper limb task to measure the effect on freezing.

Upper limb task

The upper limb task was performed before and after TBS. The task is specifically designed to elicit freezing in the upper limbs in PD patients.[4] The instruction was to make anti-phase rhythmic flexion and extension movements with the index fingers. Two different amplitudes (45^{o} [normal] or 30^{o} [small]) and two different movement frequencies (normal [100%] or fast [133%]) were used. Normal frequency was defined as the patients' specific comfortable movement speed, determined for each subject individually at the beginning of the first session. The four different conditions were: normal amplitude + normal speed (NANS), normal amplitude + fast speed (NAFS), small amplitude + normal speed (SANS) and small amplitude + fast speed (SAFS). Each condition was repeated three times per measurement (pre and post). Auditory pacing guided the first 6 movement cycles to enable the preset (100% or 133%) movement frequency at the beginning of each trial. After these first 6 movement cycles, the auditory pacing stopped and the subjects had to maintain the rhythm for 25 seconds. No visual feedback on the performance was given by covering both hands. Angular finger displacement was registered with single axis goniometers (Type F35, Biometrics Ltd., Newport, United Kingdom), which were placed over metacarpophalangeal joint of the index fingers. A calibration measurement for the goniometers was performed before each pre- and each post-measurement.

Theta burst stimulation

As stated, with TBS the excitability of neural structures can be modulated for a period that outlasts the stimulation period. TBS was administered using a MagPro figure of eight coil (C-B60, MagVenture A/S, Farum, Denmark) that was connected to a MagPro X100 stimulator. The ipsi-lateral cerebellum (1 cm below and 3 cm lateral to inion) corresponding to the most PD affected side was stimulated. TBS over the cerebellum was performed with an intensity of 70% of resting motor threshold (RMT, see next section). The two different TBS paradigms

that were used in this study are continuous TBS (cTBS) and intermittent TBS (iTBS). The basic element of cTBS is a burst of 3 stimuli at 50 Hz that is repeated every 200 ms. This pattern was repeated continuously for a period of 40 seconds (cTBS) or in 2 second trains repeated every 10 seconds for a period of 192 seconds (iTBS). Both TBS protocols consist of 600 TMS pulses.

Corticospinal excitability

With single pulse TMS, corticospinal excitability was estimated. The pulses were also administered using the above MagPro figure of eight coil that was connected to the MagPro X100 stimulator. First, the hotspot, the optimal site of the coil for eliciting motor evoked potentials (MEPs) in the resting FDI in the most affected hand, was tracked. To ensure anatomically identical coil positioning during and over the sessions, the location and orientation of the coil over the hotspot were saved as a target position using a stereotactic image guidance system (Localite TMS Navigator, Localite GmbH, Sankt Augustin, Germany). Next, the resting motor threshold (RMT) was determined, defined as the minimum stimulator intensity required to obtain MEPs with an amplitude of at least 50 μV in at least 5 out of 10 trails in the relaxed FDI of the most affected hand. Last, the minimum stimulator intensity was determined to obtain single pulse MEPs of on average 1 mV over 10 trials (SI_{1mV}). Before and after the TBS, 20 single pulses at SI_{1mV} were applied to measure the corticospinal excitability.

Data processing

The data of the goniometers were analyzed with Matlab (MathWorks, Natick, Massachusetts, USA). They were calibrated off-line with the individual calibration files. For each condition (NANS, NAFS, SANS, SAFS) the peak-to-peak and frequency values were calculated per movement cycle. In order to define each movement cycle, the turning points in movement direction (positive to negative, and vice versa) in the signal had to be detected. A peak had to meet the following three criteria: (1) the time derivative changes sign (2) the difference in absolute value between two consecutive peaks had to be at least one degree and (3) for positive peaks, the value of the peak had to be higher than the preceding and following peak. For negative peaks, the value of the peak had to be lower than the preceding and following peak. A single cycle is defined as the period between a maximum peak value and the following maximum peak value.

Freezing trials

For each pre and post measurement the number of freezing episodes per condition was defined. In accordance with Vercruysse et al.[3], the beginning of a freezing episode was determined as “the onset of abnormally small motion cycles (<50% of the initial amplitude)

accompanied by an irregular cycle frequency". The end of the freezing episode was defined as the moment where at least 2 movement cycles with regular amplitude and frequency were resumed, or at the end of a trial. To do this an automatic detection Matlab routine was used, which was visually checked. The minimal duration of a freezing episode had to be at least "75% of a normal cycle duration".

Statistical analyses

Statistical analyses were performed in SPSS (IBM SPSS Statistics 20). Paired sample t-tests were performed to test the difference between the pre and post measurement. Two freezing variables, namely total freezing duration and the number of episodes were tested for the complete upper limb task (NANS, NAFS, SANS, and SAFS together) and for the SAFS condition separately. The tests were performed for the most affected and less affected hand and cTBS and iTBS separately. A change in corticospinal excitability was tested comparing the pre and post MEP amplitudes. For all tests, $P < 0.05$ was considered significant. Data are shown as means ± standard deviation.

Results

Subjects

In total 17 PD patients (13 men) were included in the analyses. Two patients dropped out during the first session TBS. They felt uncomfortable because of co-activation of neck muscles during the TBS. One patient experienced the protocol stressful and did not participate in the second session. Clinical and demographic characteristics are listed in Table 1.

Table 1. Clinical and demographic characteristics of the 17 PD patients.

	Mean	Range
Age (years)	61.2	46 - 76
PD duration (years)	8.5	1 - 25
FOG duration (years)	3.4	1 - 12
Hoehn-Yahr	2.4	2 - 3
UPDRS	33.4	12 - 68
NFOGQ	16.5	3 - 28
FAB	16.0	12 - 18
MMSE	28.5	24 - 30
RMT (%MSO)	43	34 - 60

UPDRS = unified Parkinson's disease rating scale part 3; NFOGQ = new freezing of gait questionnaire; FAB = frontal assessment battery; MMSE = mini mental state examination; RMT = resting motor threshold.

Freezing

Figure 1 shows the effect of the cTBS protocol on freezing duration and on the number of freezing episodes in the most affected and in the less affected hand. The cTBS appeared not to have an effect on freezing of the upper limbs when all four conditions were combined (complete task), nor in any of the separate four movement conditions. In contrast, in Figure 2A it is shown that on the complete task the iTBS protocol had an effect on the freezing duration. In the most affected hand a significant decrease was found (P = 0.009) in mean FOUL duration within the task, together with a slight, but significant duration increase in the less affected hand (P = 0.036). Since the most stressful condition (SAFS) previously has shown to be most prone to detect the FOUL's,[4] the conditions were analyzed separately. In the SANS and SAFS conditions, a significant decrease in freezing duration in the most affected hand was detected (P = 0.017 and 0.029, respectively). The number of freezing episodes has not changed after iTBS (Figure 2B).

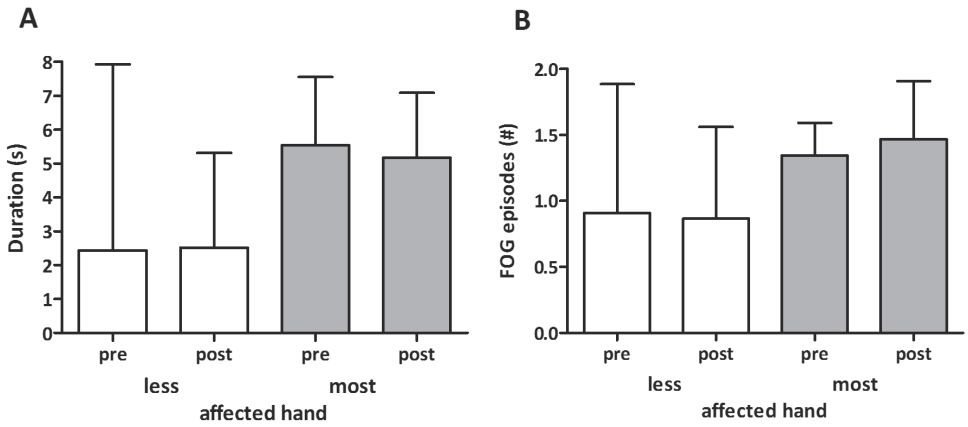

Figure 1. Inhibitory cTBS results on the mean freezing duration (A) and number of freezing episodes (B) of the complete upper limb task before and after the TBS. The white bars show the results of the less affected hand and the gray bars of the most affected hand. The error bars signify the standard deviation.

Corticospinal excitability

The cTBS over the cerebellum did not have a significant effect on the corticospinal excitability, measured over the primary motor cortex contralateral to the most affected side. There is a small trend (P = 0.130) for an excitability decrease after the iTBS protocol (Figure 3).

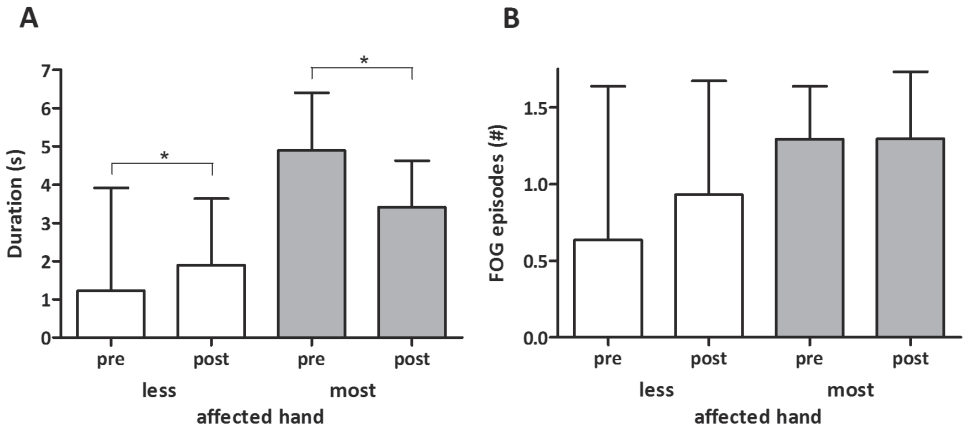

Figure 2. Excitatory iTBS results on the mean freezing duration (A) and number of freezing episodes (B) of the complete upper limb task before and after the TBS. The white bars show the results of the less affected hand and the gray bars of the most affected hand. The error bars signify the standard deviation. The asterisks indicate a significant differences between pre and post measurements.

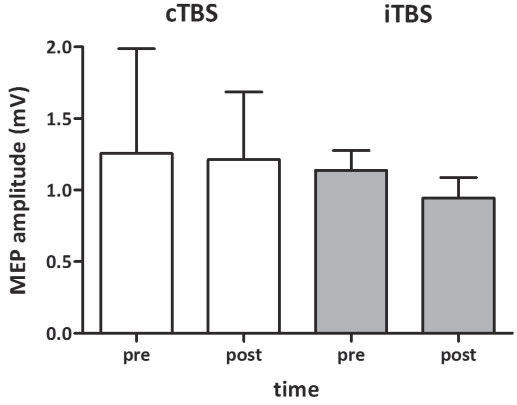

Figure 3. The amplitude of single pulse MEPs pre and post the TBS over the cerebellum. The white bars show the results of the cTBS and the gray bars of the iTBS. The error bars signify the standard deviation.

Discussion

We studied the possibility to decrease freezing of the upper limbs by stimulating the cerebellum with two opposite theta burst stimulation protocols. The iTBS protocol significantly decreased the freezing duration during the upper limb task in the most affected hand. The cTBS protocol did not show an effect on both freezing variables (total duration and number of episodes).

Koch and colleagues investigated the effect of cTBS in PD patients with levodopa-induced dyskinesia.[19] They found that a single session of cerebellar cTBS was capable of transiently reducing levodopa-induced dyskinesia in a group of patients with PD. Without claiming that FOG/FOUL and dyskinesia in PD are opposite symptoms, it is interesting that these opposite TBS protocols have a positive, i.e. also roughly opposite, effect in PD.

Vercruysse and colleagues tried to identify the neural correlates of motor blocks or "freezing episodes" during a bimanual motor task in PD patients with FOG.[26] Using functional MRI (fMRI) they found that FOUL episodes were associated with increased cortical (right SMA, dorsal premotor and primary motor cortex, and left prefrontal cortex) brain activity, while subcortical activity in the bilateral pallidum and putamen was decreased. Previous fMRI studies of upper limb motion in PD patients without FOG have consistently shown increased activation in premotor-parietal and cerebellar regions, presumably to compensate for the dysfunctional striato-supplementary motor loop.[27] They showed a compensatory shift in functional connectivity between SMA and other motor regions enhanced bimanual performance in PD, especially during antiphasic movements. It would therefore be interesting to repeat the current study with TBS over cortical areas, especially the SMA. Moreover, fMRI of both structures (cerebellum, SMA) in combination with the results of our TBS interventions might further increase our understanding.

In our study, no significant changes in MEP amplitude in the task relevant FDI muscle were measured after the TBS over the cerebellum. In healthy subjects, Popa et al.[17] did not observe changes in corticospinal excitability after cTBS and iTBS, whereas Koch et al.[16] found an excitability decrease after cTBS and an increase after iTBS. An explanation for different result in healthy subjects could be just an explanation of our results: since PD patients show a different brain activity pattern than healthy controls, also the cerebellar-motor cortex connection is aberrant.

Vercruysse and colleagues concluded that FOUL mostly occurs when the motor system is stressed, for example, by imposing small and fast finger movements, rather than by finger movements at comfortable pace and amplitude.[3] In our patients we could confirm this observation as that the SANS and SAFS conditions provoked longer freezing episodes.

We proposed in our introduction that a boosting of the cerebellar activity might *a priori* not be caused by a boosting iTBS protocol, but by a protocol like cTBS that inhibits the, themselves inhibiting, Purkinje cells in the superficial cerebellar layer. This could also have led to an increase of freezing following iTBS. This was not what we found. Continuous TBS had no effect whatsoever and with iTBS freezing diminished. That even a negative effect did occur for the less affected hand can be seen as a confirmation of the importance and the deviant, probably compensating, role of the cerebellar function at the affected side. Also here, a deeper understanding of the mechanisms at hand would need a functional imaging technique like fMRI.

In conclusion, the findings presented here support the hypothesis about the compensatory mechanism of the cerebellar activity in PD patients without FOG and that stimulating the cerebellum in patients with FOG decreases freezing. To clearify the neuronal mechanisms and pathways behind this compensation further research is needed in the form of (f)MRI studies.

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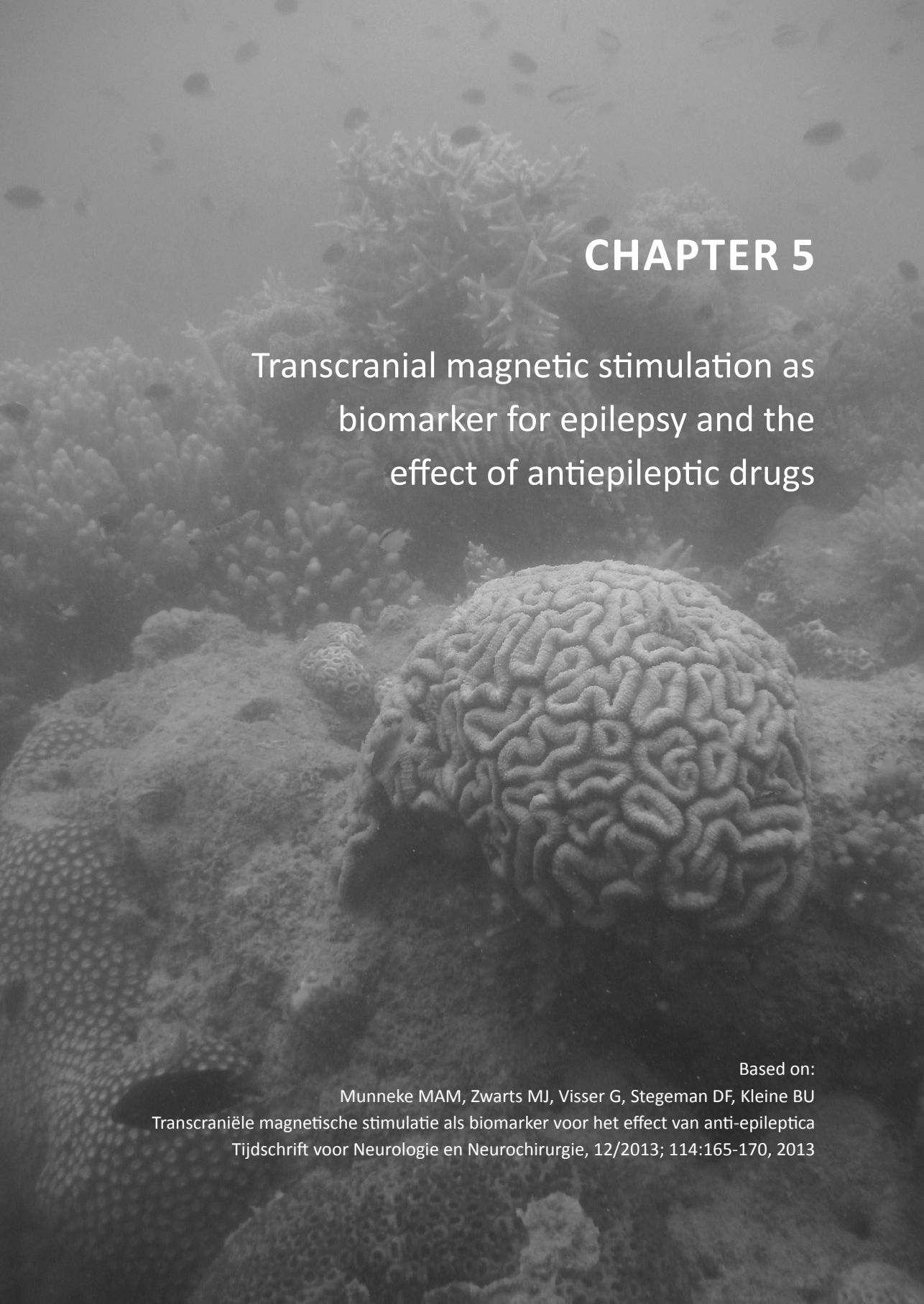

CHAPTER 5

Transcranial magnetic stimulation as biomarker for epilepsy and the effect of antiepileptic drugs

Based on:
Munneke MAM, Zwarts MJ, Visser G, Stegeman DF, Kleine BU
Transcraniële magnetische stimulatie als biomarker voor het effect van anti-epileptica
Tijdschrift voor Neurologie en Neurochirurgie, 12/2013; 114:165-170, 2013

Abstract

Abnormal excitation and excitability of the cerebral cortex are characteristics of epilepsy. A direct way to measure excitability is transcranial magnetic stimulation (TMS). In patients with epilepsy, motor evoked potentials can be recorded safely, and serve as a maker for cortical excitability in general. In healthy subjects, the effects of a single dose of an antiepileptic drug on excitability are related to the mechanism of its action. In epilepsy, TMS provides information about the epileptic network and the effect of medication thereon. Improved intracortical inhibition shortly after starting an antiepileptic drug is predictive of the clinical response in the next year.

Introduction

Abnormal increased excitation and excitability of the cerebral cortex are fundamental characteristics of epilepsy. Interictal epileptiform activity in the EEG reflects this indirectly. It is possible to measure excitability in a more direct way by using transcranial magnetic stimulation (TMS). The diagnostic application of magnetic stimulation started with the magnetic stimulator demonstrated by Barker and colleagues in 1985 [1].

So far, much of the clinical TMS research has focused primarily on conditions of the motor system and, to a lesser extent on abnormal brain states as for instance in epilepsy. In this review, measurement of excitability will be described first. This is followed by a summary of the studies in epilepsy carried out so far, and the effects of antiepileptic drugs. In this, motor excitability will be considered as representative of cortical physiology and pharmacology in general. The aim is to discuss the use of motor evoked potentials as a marker for the excitability of the epileptic network and its suppression by anti-epileptic drugs.

Cortical stimulation and motor-evoked potentials (MEPs)

The basic principle of TMS is electromagnetic induction. The device sends a brief strong current through a coil, resulting in a rapidly changing magnetic field. In electrically conductive tissue, including the brain, the magnetic field induces an electric current which can activate (depolarize) axons. Stimulation of the motor cortex may depolarize axons of the pyramidal tract directly indirectly through cortical interneurons. Indirect means that axons of cortical interneurons act through intermediate synaptic connections. This direct or indirect activation of the pyramidal tract ultimately results in a muscle contraction. The concomitant electrical muscle activity, the motor-evoked potential (MEP), can be measured and quantified with electromyography (EMG). The amplitude of the MEPs varies between successive stimuli. This is explained by the fact that most of the evoked motor activity is attributable to the indirect activation of pyramidal tract neurons via the cortical interneurons. The synaptic transmission may be modulated by the state of the motor networks or by the action of antiepileptic drugs [2].

Since TMS was first used, adverse effects have been reported and an international consensus group has been set up to draw up guidelines for the use of TMS [3]. In the past, patients with epilepsy were usually excluded from studies using TMS. The assumption was that TMS could provoke epileptic seizures. It has been found, however, that only relatively high-frequency repetitive TMS protocols may provoke a seizure [3]. This is not the case when TMS with single or double pulses is used such as described below. A number of seizures have indeed been described in studies in epileptic patients, but, at closer inspection, these do not appear to

have been be provoked by TMS. The occurrence of a seizure during the TMS study could, in such studies, in patients with refractory epilepsy be explained by the risk of a coincidental seizure [4].

Motor threshold, excitability and paired-pulse TMS

In contrast to studies of central motor conduction, studies of cortical excitability look only at the amplitude of the MEP. Due to the variation described above, an average is determined over, for example, 10 stimulations. For the entire study tens to hundreds of stimuli are therefore needed. However, due to the relatively low intensity of stimulation and the limited muscle contractions provoked, most patients and test subjects tolerate the investigation very well [5].
In a standard TMS study the motor threshold is determined at rest (resting motor threshold, rMT). By definition this is the TMS intensity which causes an MEP of a minimum of 100 µV in half of the measurements (5 out of 10) [6]. For other parts of the study stimulus intensities are expressed as a percentage of this motor threshold. It is very important that this threshold is determined for each individual and for each hemisphere, because the excitability can vary significantly due to, amongst other things, the difference in distance from coil to motor cortex. Changes in excitability as a result of medication or other interventions can be measured only as a relative change within one individual subject or patient.
In the case of epilepsy the so-called “paired-pulse” TMS protocols are relevant Two stimuli in rapid succession are given: a conditioning stimulus followed by a test stimulus (Figure 1). The intensity of the conditioning stimulus is almost always below the motor threshold, whereas the test stimulus is always above that threshold. The MEP amplitude after a paired pulse is expressed as a percentage of the response to the test stimulus alone [7]. The conditioning stimulus is, if below the motor threshold, by definition not sufficient to provoke an MEP, but sufficient to activate cortical (inter)neurons. The test stimulus is used to examine what the first stimulus brings about in the network. A short interstimulus interval of 2-3 ms causes intracortical inhibition. With an interval of 5 to 20 ms, intracortical facilitation occurs and thus an increased MEP in response to the test stimulus. Upon further increasing the interval to between 50 and 300 ms, inhibition is again observed.

Paired-pulse TMS has been investigated in various patients groups. A striking finding in several neurological conditions is a decrease in intracortical inhibition [8,9]. For this inhibition, GABAergic neurones are needed that may be disturbed by neurotransmitter or ion-channel disorders, as is the case in epilepsy.

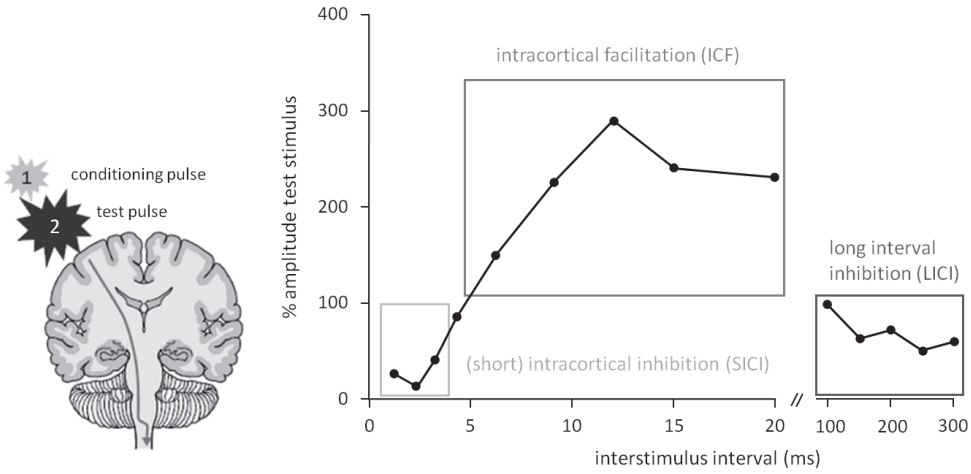

Figure 1. Schematic representation of the paired-pulse TMS method. A conditioning stimulus precedes the test stimulus. For the short interstimulus intervals a subthreshold conditioning stimulus is used (80% rMT) followed by a test stimulus (120% rMT). For the long intervals (> 20 ms) both stimuli are above threshold (120% rMT). The graph shows the relation between the interstimulus interval and MEP amplitude (as a percentage of the test MEP amplitude). Adapted figure from Lancet Neurology [24] © 2003, with permission from Elsevier.

Antiepileptic drugs and excitability

Shortly after the paired-pulse protocol was described, pharmacological studies were carried out. Initially these focused on the question of which synaptic mechanisms are responsible for intracortical inhibition and facilitation. Various antiepileptic drugs were also investigated in these studies [10]. In most studies the effect of a single dose in healthy subjects was investigated. The effects observed shortly after medication administration can be summarized as follows [11]:

- The classic sodium channel blockers phenytoin, carbamazepine and lamotrigine increase the motor threshold. However, if the study protocol corrects for the change in threshold, the pattern of intracortical inhibition and excitation does not change when interstimulus intervals change. Recently a similar result was also found for lacosamide [12].
- In contrast, the $GABA_A$ receptor agonists lorazepam, diazepam, topiramate, gabapentin and pregabaline enhance intracortical inhibition, without a change in threshold. These findings are consistent with the mechanism of direct and indirect influence on GABAergic transmission.
- Antiepileptic drugs with different mechanisms of action show a range of TMS effects. Valproic acid changes neither the threshold nor the paired-pulse results. For levetiracetam initially no effects on TMS measurements were found either [13]. However, one large study showed a slight decrease in motor threshold [14].

The effects of long-term use of antiepileptic drugs have hardly been investigated. Lee and colleagues [15] investigated healthy subjects during a three-week titration phase and a five-week maintenance phase of carbamazepine or lamotrigine. Repeated TMS measurements showed a concentration-dependent increase in the threshold, comparable to the effects of short-term administration. In addition, enhanced intracortical inhibition was also observed with both treatments. It is not clear what causes this additional effect of chronic administration and which synapses are involved. On sudden discontinuation of the medication the return of intracortical inhibition follows the course of the blood levels, while, in the majority of subjects the effect on the threshold persists somewhat longer. However, in a minority of subjects a reduction in the motor threshold, even to below the baseline value, is seen shortly after discontinuation of carbamazepine. This rebound effect could explain the disruption in seizure control when medication is withdrawn too rapidly.

Epilepsy and excitability

Changes in levels of cortical excitability in idiopathic generalized epilepsy (IGE) were first described by Reutens and colleagues [16]. They found a reduction in the motor threshold in untreated patients. Upon treatment with valproic acid, normalization of the TMS results was seen. The effects of valproic acid in patients are therefore different compared to those in healthy subjects. On the one hand, this can be explained by chronic administration; on the other hand, one may assume a different initial state of the epileptic network.

More recently Badawy and colleagues [17] described TMS in a group of 199 patients with a first epileptic seizure or with newly diagnosed epilepsy. In all patients excitability was investigated with motor threshold and paired-pulse TMS. A proportion of the patients were examined longitudinally up to four times. In interictal measurements in patients with IGE a (slight) reduction of the motor threshold and a decrease in intracortical inhibition were seen with several short and long interstimulus intervals in both hemispheres. In focal epilepsy there was decreased inhibition of the affected hemisphere only. The inhibition in the unaffected hemisphere was similar to that in healthy subjects. The changes in excitability were dependent on the time of measurement. In the 24 hours prior to a seizure a reduction in motor threshold was found, while, in contrast, during the 24 hours after a seizure the motor threshold was raised. This pattern appears to be the same for all tonic-clonic seizures, regardless of whether they were focal or generalized at onset. In focal epilepsy the finding of abnormal excitability in the contralateral hemisphere depends on the extent of its involvement in secondary generalization of the seizure investigated [18].

Sleep deprivation can disrupt intracortical inhibition and reduces the motor threshold slightly. This effect is strongest in juvenile myoclonic epilepsy and less pronounced in other epilepsy syndromes [19,20]. TMS has also been performed in healthy siblings of patients with epilepsy. Compared to healthy controls without a family member with epilepsy, intracortical inhibition at intervals of 250 and 300 ms was reduced. Less than normal intracortical inhibition was found in families with generalized epilepsy and also in families with focal epilepsy. The presence of this TMS pattern even in siblings of patients with lesional epilepsy supports the concept of some involvement of genetic factors in all epilepsies [21].

Effect of anti-epileptic drugs on excitability

In the patient cohort studied by Badawy and colleagues [22] the TMS measurement was repeated after commencement of an antiepileptic drug therapy. In IGE valproic acid was prescribed predominantly, in focal epilepsy mainly carbamazepine. In both groups a proportion of the patients were treated with lamotrigine monotherapy. The TMS findings after commencement of the medication (8 weeks on average) were compared with the baseline measurement and were related to the clinical course after one year. In those patients who remained free of seizures for one year, there was an increase in the motor threshold. There was an even stronger association between a good response to the first antiepileptic drug and normalization of intracortical inhibition. With an interstimulus interval of 250 ms the contrast between responders and non-responders was most apparent (Figure 2). In patients with idiopathic generalized epilepsy, an increase in inhibition between the baseline measurement and the measurement with medication has a predictive value of 97% for the subsequent year being seizure-free. In focal epilepsy the positive predictive value is lower at 69%. This should be compared with the a priori probability of being seizure-free with medication; in this case 69% in IGE and 59% in focal epilepsy. The negative predictive value is, when using the same cut-off value of 100% change in intracortical inhibition, lower: 42% and 45% respectively. Upon visual assessment of the data (see images in the article by Badawy et al. [22]) it seems that, by selecting a different cut-off value, a better prediction about the failure of an antiepileptic drug can be given. Thus, replication of these findings in a subsequent independent clinical study, preferably with cut-offs selected prospectively, is desirable and promising.

Patients with an inadequate clinical response to monotherapy were treated with a combination of two drugs. In this case too, a change in intracortical inhibition was associated with the clinical response [17].

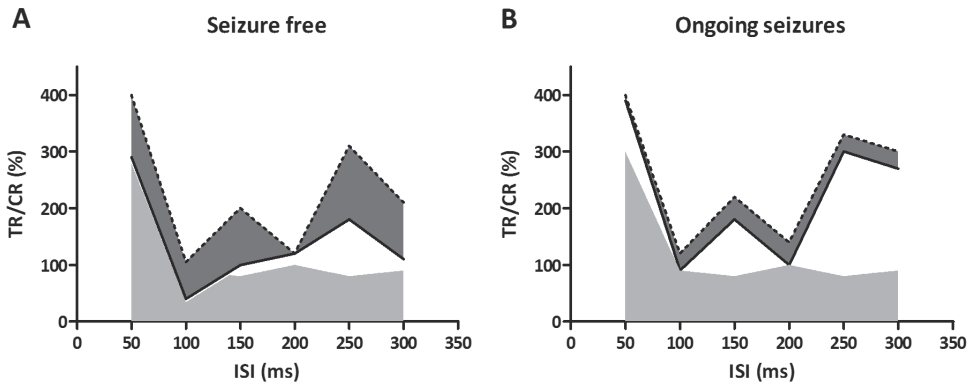

Figure 2. Pre- and post-medication long interstimulus interval curves for seizure-free patients (A) and patients with persistent seizures while on medication (B), in idiopathic generalized epilepsy (measured over the dominant hemisphere). A ratio (% amplitude response test) less than 100% means inhibition and above 100% means facilitation. The upper limit of the light gray shaded areas indicate the mean of healthy subjects. The dark gray shaded areas represent the difference before and after starting medication in the patients, and the dotted line (top) for medication administration, the continuous line (bottom) after starting medication. Adapted figure based on Annals of Neurology [22] © 2010, with permission from Wiley. Refer to source for information about the distribution.

It appears therefore that the changes in cortical excitability caused by antiepileptic drugs may allow a prediction about the clinical effect of the medication on the seizures. Especially in patients with a seizure frequency of once every few months or with even longer seizure intervals, it may take several years before the right medication is found. This can be explained simply as the result of a lack of decision points: before being able to make a statement about antiepileptic effectiveness, one must wait at least till the next seizure or the absence of seizures for 3-6 times the average pre-treatment seizure interval [23]. Further research should show whether, by using TMS, this waiting period can be shortened and a rational and reliable prediction can be made about the efficacy of the drug that has been started.

Conclusion

This review has given a summary of the literature about TMS of the motor cortex and epilepsy. In both focal and generalized epilepsy, abnormal excitability with decreased intracortical inhibition is found. This can be seen as a characteristic of the entire epilepsy network. The differences between healthy subjects and patients on the one hand, and between different epilepsy syndromes on the other hand, are too small for diagnostic purposes. Repeated measurements in the same patient may, however, provide useful prognostic information. The predictive value of a change in intracortical inhibition, measured at 250 ms, may be

used to provide more rational treatment with antiepileptic drugs. Clinical studies should show whether an unchanged inhibition in studies using TMS is predictive of the failure of a drug, so that one does not need to wait for a subsequent seizure in order to adjust the medication.

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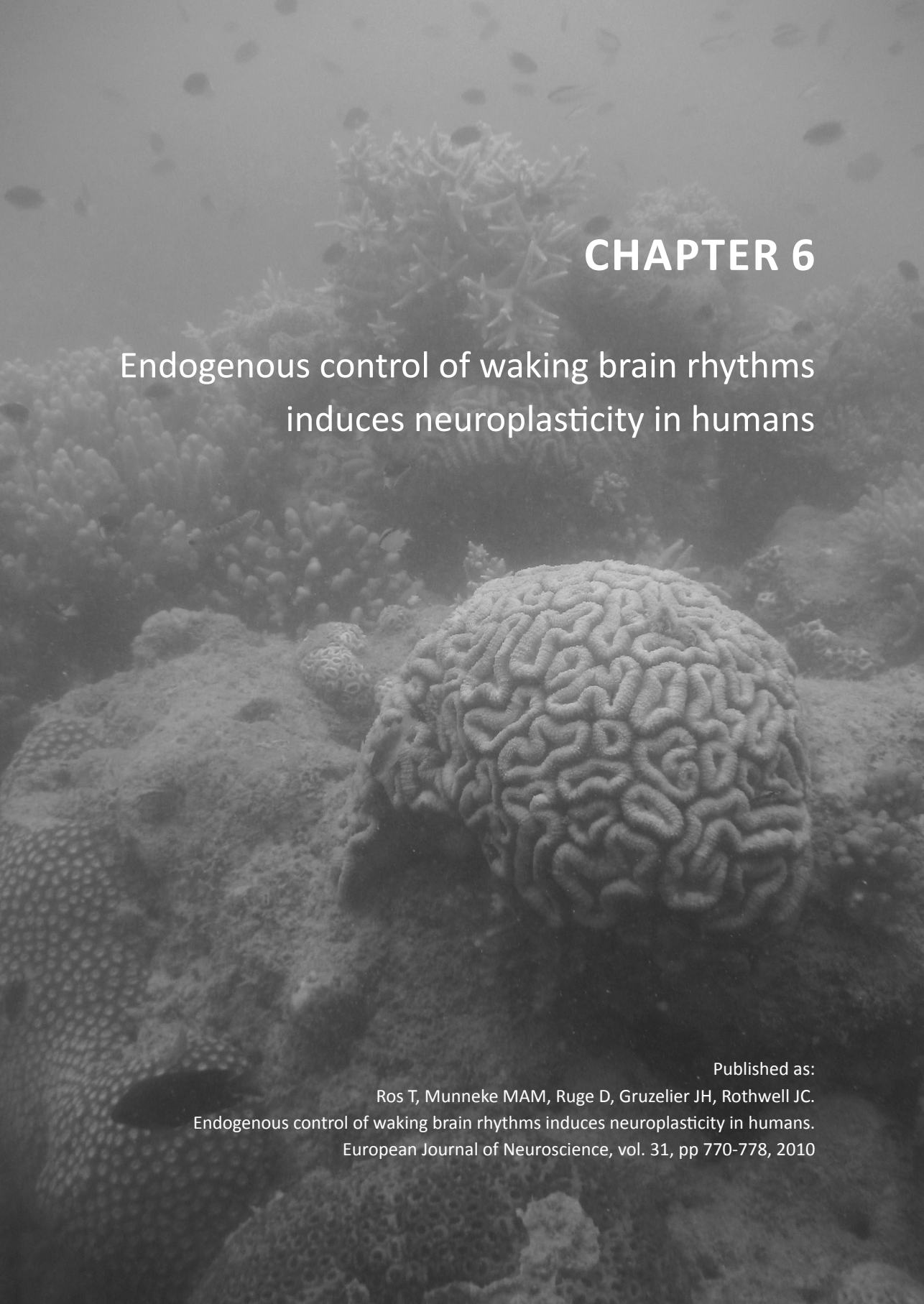

CHAPTER 6

Endogenous control of waking brain rhythms induces neuroplasticity in humans

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Abstract

We explored the possibility of noninvasively inducing long term changes in human corticomotor excitability by means of a brain-computer interface (BCI), which enables users to exert internal control over the cortical rhythms recorded from the scalp. Here we demonstrate that self-regulation of electroencephalogram rhythms in quietly sitting, naive humans significantly affects the subsequent corticomotor response to transcranial magnetic stimulation, producing durable and correlated changes in neurotransmission. Specifically, we show that the intrinsic suppression of alpha cortical rhythms can in itself produce robust increases in corticospinal excitability and decreases in intracortical inhibition of up to 150%, which last for at least 20 minutes. Our observations may have important implications for therapies of brain disorders associated with abnormal cortical rhythms, and support the use of EEG-based neurofeedback as a noninvasive tool for establishing a causal link between rhythmic cortical activities and their functions.

Introduction

Brain oscillations have thus far been implicated in many 'ongoing' functions such as binding and attention [1-3], however less direct evidence exists on the long-term effects of their entrainment and possible role in brain plasticity [4]. Today's brain stimulation devices, including transcranial magnetic stimulation (TMS) and direct-current stimulation (tDCS), are noninvasive and enable the accurate study of neuroplasticity in the intact human brain. The added hope is that their joint use with electroencephalogram (EEG) registration will further elucidate the functions of neuronal oscillations.

The present study, which combines TMS and EEG, is the first to additionally exploit a brain-computer interface (BCI) in order to manipulate brain rhythms endogenously [5]. A BCI allows real-time information of brain activity to be fed-back to a user by means of a computer in a closed "neurofeedback" loop (NFB), enabling endogenous control and natural operation of brain oscillations across cortical networks *in vivo* [6,7]. Crucially, brain stimulation investigations to date have induced plasticity by magnetic or electric fields that are by definition exogenous and artificial. Such patterns and the driving forces they produce may not necessarily be intrinsic to the brain. Moreover, the inherent problem faced by many behavioural manipulations of the EEG is the difficulty of dissociating stimulus-dependent versus stimulus-independent oscillations. During NFB subjects are exposed to the same visual feedback stimuli, hence their entrained EEG differences may be considered as resulting minimally from external factors, and instead represent the modulation of internal or 'background' brain state(s). Finally, we also investigated the relation between TMS measures and the full-band EEG [8], which here includes very fast oscillations (>100 Hz), as well as slow direct currents (DC). To the best of our knowledge, specific changes in these two latter EEG measures have not been previously explored in TMS-EEG studies to date [9].

While neuroplasticity appears to be active through diverse cellular processes [10] in the central nervous system, in TMS methodology it is operationally defined as a significant and lasting change in the motor evoked potential (MEP), whose amplitude is representative of the strength of neurotransmission from motor cortex to muscle, evoked by a magnetic pulse. We therefore asked whether both *pronounced* and *persistent* oscillatory patterns expressed during NFB would be associated with tangible and long-lasting (plastic) changes in MEPs elicited by TMS over the primary motor cortex. A growing body of evidence [11] indicates that MEPs evoked by single TMS pulses best reflect the overall responsiveness of the corticospinal pathway, or corticospinal excitability (CSE), whereas those originating from paired pulses enable the discrimination of intracortical transynaptic mechanisms, such as those pertaining to short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). Our hypothesis was that NFB-induced alpha (8-12 Hz) rhythm desynchronisation, generally considered a marker of cortical activation [12], might produce a durable enhancement

in corticospinal excitability, given that previous studies have found an inverse association between spontaneous alpha synchronisation and MEP amplitude [13,14]. In contrast, low beta (12-15 Hz) synchronisation, which has been associated with cortical deactivation [15] and motor inhibition [16], might produce an opposite pattern.

Methods

Study design

24 healthy participants (12 women, age: 31 ± 5 years, all right-handed), were randomly allocated to 2 protocol groups for a single 30-min NFB session: alpha desynchronisation (*n* = 12) or low beta *synchronisation (n = 12).* All participants were naive to the neurofeedback protocols used in this study. Experimental procedures were approved by the local ethics committee in accordance with the Declaration of Helsinki, and no adverse effects were reported by the participants during the study.

Neurofeedback (NFB) apparatus and procedure

EEG signals were recorded using a NeXus-10 DC-coupled EEG amplifier using a 24-bit A-D converter (MindMedia, Netherlands) capable of full-band EEG recording, and NFB training was carried out with Biotrace+ software on an Intel DualCore computer with a 15" screen. The EEG used for recording and feedback was sampled at 256 Hz with an Ag/Cl scalp electrode placed above the right first dorsal interosseous (FDI) muscle cortical representation/'hot spot' (approx. C3), which was referenced to the contralateral mastoid. The scalp area was carefully scrubbed with NuPrep abrasive gel, followed by application of Ten20 electrode paste. The ground electrode was placed on the right arm. For the purpose of online NFB training, the EEG signal was infinite impulse response (IIR) bandpass filtered to extract alpha (8-12 Hz) and low beta (12-15) amplitudes (μV peak-to-peak) with an epoch size of 0.5 seconds. Likewise, EEG was passively co-registered at the left FDI motor cortical representation (approx. C4) referenced to its contralateral mastoid. In order to analyse data offline, IIR digital filtered (Butterworth 3rd order) EEG amplitude data of each bandwidth (DC, delta (1-4 Hz), theta (4-7Hz), alpha (8-12 Hz), low beta (12-15 Hz), beta (15-25 Hz), high beta (25-40 Hz), low gamma (40-60 Hz), and high gamma (60-120 Hz) was then exported at 32 samples/second. In addition, offline Fast Fourier Transform (FFT) of raw (256 Hz) data was used calculate and export the *mean frequency* for each bandwidth (except for DC) at 32 samples/second. All sampled data was subject to offline voltage-threshold artifacting for ocular, head movement and muscle contamination, whereby outlying data points with amplitudes of >3 standard deviations were rejected using histogram analysis of each bandwidth. All means were then computed for the 3 minute epochs each defined as a 'period'. Periods 0 and 11 consisted of

pre and post (feedback-free) resting EEG measurements in the eyes open condition. Periods 1-10 consisted of visual feedback training.

Neurofeedback training procedures

The first resting baseline was recorded during a 3-min eyes open EEG recording at rest just before the start of feedback, and the second 3-min just after the end of training. During feedback, the ALPHA group aimed to suppress absolute alpha (8-12 Hz) amplitude while the BETA group aimed to elevate absolute low beta amplitude (12-15 Hz). Subjects were given no explicit instructions or mental strategies by the experimenter on how to achieve control over their EEG, but were told to be guided by the visual feedback process. This consisted of a clearly visible bar graph on the left hand side of the screen whose height was proportional and fluctuated according to the real-time amplitude of the relevant scalp EEG rhythm. Participants were told to try and learn to maintain the level of the bar graph for as long as possible either above (in case of low beta) or below (in case of alpha) a set threshold. This threshold was automatically computed and set to be either 30% of the time above or below the initial 3-minute mean baseline alpha or low beta amplitude, respectively. The dynamic of several visual games could thus be influenced depending on the volitional control of the EEG amplitude and whether the "reward" threshold condition was met. For example, in a game called 'Puzzles', moving puzzles automatically assembled to form an image but this process would momentarily stop when the reward threshold was not met during feedback. All other games were based on a similar "start/stop" scenario, and included the 'Mazeman', 'Space Invaders', 'Mandala' and 'Bugs' games which are part of the Biotrace+ software (MindMedia, Netherlands). Both NFB protocols used the same series of displays and games, which were given in a random order for approx. 6 minutes each. For the low beta protocol a supplementary inhibit (40-60 Hz) that temporarily stopped the game was used to ensure low beta reward was not muscle artifact driven. Right (FDI) and left (FDI) hand electromyographic (EMG) activity was monitored via the EMG amplifier used to record the TMS motor evoked potentials.

Neurofeedback data analyses

Offline analysis of NFB training efficacy for each subject was defined by a *training coefficient*, or the Pearson correlation between the period number (0 to 10, baseline = 0) and the average EEG amplitude (μV, peak-to-peak) of that period. This had a range of -1 (relative decrease) to +1 (relative increase). Hence for subjects in the ALPHA and BETA groups successful training was indicated by more negative or positive coefficients, respectively. Additionally, the normalised training EEG change for each subject was estimated by the ratio of the average EEG amplitude for each of the 10 training periods and the first baseline EEG, and designated as training EEG change (for that period). Likewise, the normalised change

in the baseline EEG amplitude was expressed by the ratio of the second divided by the first baseline, and designated as resting EEG change.

Transcranial magnetic stimulation (TMS) apparatus and procedure

The course of the experiment is shown in Figure 1, which was used to test the impact of NFB training on corticomotor measures of corticospinal excitability (CSE), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF). TMS parameters (CSE, SICI, and ICF) were measured before (T_0) and twice after NFB (T_1 and T_2). In random order, 78 TMS responses were measured, which required approximately 6 minutes per hemisphere. We evaluated the TMS parameters of both hemispheres, first left (trained) and then right (untrained) hemisphere, to investigate hemispheric effects of NFB. The T_1 measurements were performed circa 3-15 minutes after NFB training, and T_2 after 15-27 minutes. Well established standard TMS paradigms were used to measure the corticospinal and intracortical parameters [11]. All measurements were carried out with two monophasic Magstim 200 magnetic stimulators (Magstim, Whitland, UK), which were connected with a "Y-cable" to a 70 mm figure-of-eight coil. We determined the 'hot spot' of the first dorsal interosseous muscles (FDI) for each hemisphere separately. The coil was placed flat on the skull with the handle pointing backward and rotated about 45° away from the midline. Resting motor threshold (RMT) intensity was defined as the lowest stimulator output intensity capable of inducing motor evoked potentials (MEPs) of at least 50 μV peak-to-peak amplitude in the FDI muscle in at least half of 10 trials. Active motor threshold (AMT) was defined as the intensity needed to evoke an MEP of about 200 μV during a 5-10% maximum voluntary contraction. Corticospinal excitability (CSE) was quantified by the amplitude of the motor evoked potential (MEP) elicited by a *single* test TMS pulse. The test pulse intensity was set to yield an average MEP amplitude of 1 mV at baseline (T_0), and was kept constant throughout the experiment. Short interval intracortical inhibition and intracortical facilitation (SICI and ICF) were evaluated using the paired pulse protocol developed by Kujirai et al (1993). In random trials the test pulse was preceded by a subthreshold conditioning pulse (80% AMT) with an interstimulus interval (ISI) of 2, 3, 10 or 12 ms. The test response was suppressed (SICI) at ISI=3ms; whereas facilitation occurred at ISI=10 and 12ms (ICF = mean of both time points). A run consisted of 78 stimuli given at approximately 0.25 Hz. 48 paired-pulse (12 for each ISI) and 30 single-pulse MEPs were recorded. Single-pulse MEP amplitudes were normalised as T_1 divided by T_0, and T_2 divided by T_0, respectively. For SICI and ICF the amplitude of the conditioned response was expressed as a percent of the amplitude of the test response alone. Ratios < 1 indicate inhibition, whereas ratios > 1 indicate facilitation.

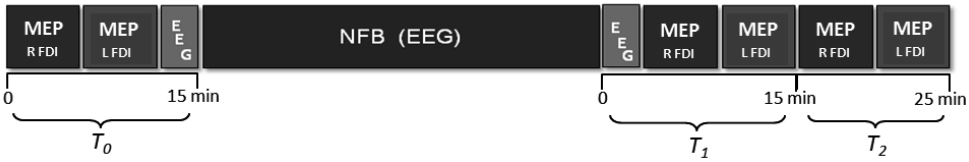

Figure 1. Schema showing time-line of the experiment. Before and twice after neurofeedback training (NFB), motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) were recorded during 6-min blocks of time periods T_0 ,T_1 and T_2 from the hand muscles corresponding to the trained left and untrained right hemisphere corticospinal projections.

EMG measures and analysis

Surface electromyographic (EMG) recordings were made using a belly-tendon montage with Ag/AgCl-plated surface electrodes (9 mm diameter). Raw EMG signal was amplified and filtered using Digitimer D150 amplifiers (Digitimer Ltd., Welwyn Garden City, Herts., UK), with a time constant of 3 ms and a low-pass filter of 3 kHz. Signals were recorded via a CED 1401 laboratory interface (Cambridge Electronic Design Ltd., Cambridge, UK) and stored on a PC for later analysis using a sampling rate of 5 kHz.

Statistical analyses

All statistical procedures were two-tailed with significance set at $\alpha = 0.05$. Protocol group EEG differences were examined with a GROUP x PERIODS (2 x 11) repeated measures ANOVA, from period 0 (baseline) to period 10. Within-group EEG was assessed by a one-way repeated measures ANOVA with PERIODS as a factor; post hoc Dunnett's test was used to detect significant changes from the baseline rest period. TMS measures of CSE, SICI, and ICF for each hemisphere were separately subjected to a GROUP x TIME (2 x 3) repeated measures ANOVA; Greenhouse-Geisser correction was used where necessary. Subsequent to reliable main effects, planned comparisons were conducted by Bonferroni corrected t-tests for long-term (>20 min) changes after NFB (T_0 - T_2). A regression analysis was performed between normalised EEG (%baseline) vs. normalised TMS parameters (%baseline), as well as between training vs resting EEG (%baseline). With regards to the weighted least squares (WLS) regression analysis, the reciprocal variance of the relevant training period amplitude (32 samples/sec) was used as each subject's weighting factor. Statistical analyses and structural equation modelling (SEM) were respectively carried out with SPSS 15.0 and Amos v7.0 (SPSS Inc., Chicago, IL, USA). For SEM we used maximum-likelihood estimation as well as bootstrapping (2000 samples, with a 95 % bias-corrected confidence level). The final indirect model was also verified by an automatic specification search in the software. Chi-square (CMIN) and baseline fit measures (e.g. NFI) were used to estimate relative goodness-of-fit, along with parsimony measures (e.g. PNFI).

Results

NFB is associated with significant changes in EEG amplitude during training

ALPHA and BETA protocol subjects attempted to respectively decrease their alpha or increase their low beta EEG amplitudes, recorded from left motor cortex during a 30-min NFB training session; which, for the sake of analysis, was subdivided into 10 equal segments of 3-min each, called 'periods'. A feedback-free, eyes-open, resting baseline was also recorded for 3-min (period 0) before the start and after the end of NFB. A repeated measures one-way ANOVA on the ALPHA-group revealed that alpha amplitude in the trained hemisphere decreased significantly ($F(10,110) = 2.7$, $p < 0.05$) from baseline (9.08) to period 10 (8.50), with a largest decrement at 15-18 minutes, or period 6 (7.93, $t_{11} = 4.0$, $p < 0.01$). As seen in Figure 2A, for the trained hemisphere, post-hoc Dunnett's test comparisons with the baseline period revealed a significant reduction ($p < 0.05$) for all periods except periods 2, 8, and 10. Interestingly, high gamma *mean frequency* (60-120 Hz) was inversely correlated with alpha amplitude during training ($r = -0.25$, $p < 0.01$). Within-subject amplitude correlations between theta, alpha, low beta, and high beta during NFB were consistently positive within a statistically significant range of $0.5 < r < 0.9$ ($p < 0.01$). No reliable associations were detected between oscillatory EEG bands and direct current (DC) shifts, although the latter exhibited a negative correlation with period number ($r = -0.31$, $p < 0.01$). In contrast, as seen in Figure 2B, one-way ANOVA for the BETA-group trained hemisphere showed no consistent change in low beta ($F(10,110) = 1.7$, n.s.) or other EEG amplitudes.

In conclusion, NFB led to a sustained reduction in the amplitude of alpha, but not beta rhythms in naive subjects. These effects were directly associated with an increase in frequency of high gamma rhythms, and indirectly with a negative drift in DC potentials.

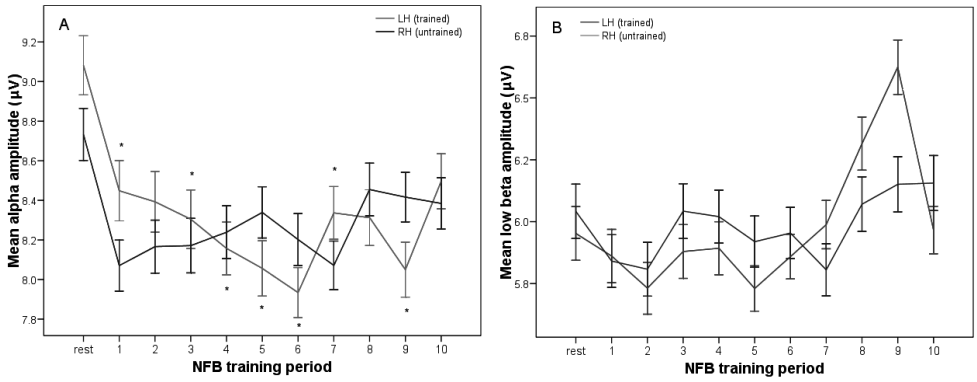

Figure 2. Time-course of the mean training EEG amplitudes for (A) ALPHA and (B) BETA groups, during a session of NFB. Each session began with a 3-min baseline at rest, followed by 30-min of EEG feedback training (periods 1-10) on the left hemisphere (LH). Right hemisphere (RH) amplitudes are also shown for the untrained hemisphere. Periods significantly different from baseline (asterisk). Error bars represent SEM.

Corticospinal and intracortical TMS measures are modified following NFB training

We measured corticospinal excitability (CSE) and short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) before (T_0) and after NFB (T_1, ~ 10 min; T_2, ~20 min). A GROUP x TIME (2 x 3) repeated measures ANOVA for the *trained* hemisphere CSE revealed a significant main effect of TIME for CSE ($F(2,44) = 6.8$, $p < 0.01$) and SICI ($F(2,44) = -4.3$, $p = 0.03$), but not for ICF ($F(2,44) = 1.6$, $p = 0.2$). Interaction effects were not significant. *No significant main effects were detected for the untrained hemisphere.* For the ALPHA group Bonferroni corrected t-tests on the trained hemisphere (Figure 3A) showed a significantly enhanced CSE at T_2 compared to T_0 (130%, $t_{11} = -2.6$, $p = 0.05$), or up to 20 min after termination of NFB training. In the trained hemisphere only, we observed a significant correlation between TIME and MEP amplitude ($r = 0.43$, $p < 0.01$). In addition, as shown in Figure 4A, there was a significant decrease in short interval intracortical inhibition (SICI) in the trained hemisphere at T_2 (60%, $t_{11} = -2.6$, $p < 0.05$). Following the BETA protocol, planned t-tests in the trained hemisphere revealed no significant long-term (>20 min) changes in CSE ($t_{11} = -1.4$, $p = 0.36$) or SICI ($t_{11} = -0.6$, $p = 0.9$) at T_2. Changes in CSE and SICI in the *untrained* hemisphere are displayed in Fig. 3B and 4B respectively, revealing no significant changes for both protocols. Lastly, resting motor threshold (RMT) of the trained hemisphere was not significantly altered in the ALPHA ($t_{11} = -0.5$, n.s.) nor in the BETA group ($t_{11} = 0.6$, n.s) pre-post NFB.

Overall, significant pre-post changes in TMS measures were present only in the trained hemisphere of the alpha desynchronisation group: corticospinal excitability increased whereas intracortical inhibition decreased for at least 20 minutes after NFB.

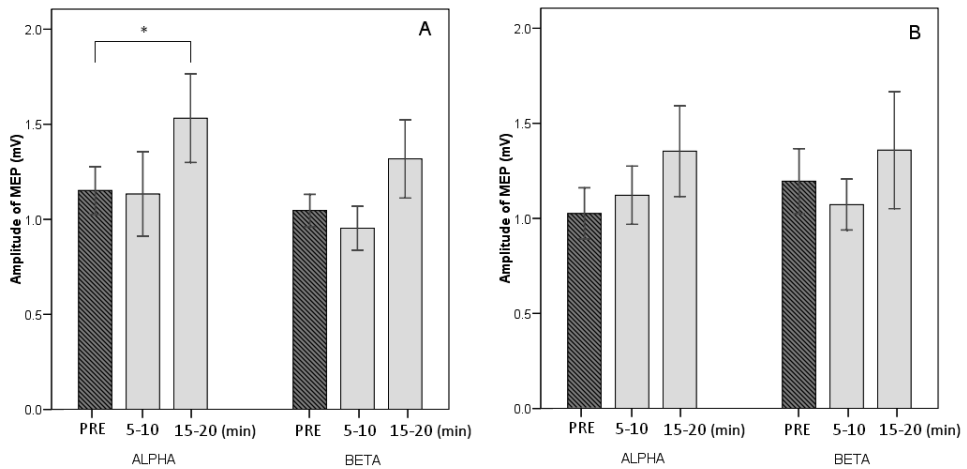

Figure 3. Mean trained hemisphere corticospinal excitability (CSE; A) and short intracortical inhibition (SICI; B) 5-10 min (or T_1) and 15-20 min (or T_2) after the ALPHA or BETA protocol. In figure B, higher MEP values indicate disinhibition (reduced SICI). Error bars represent SEM. Time periods significantly different from PRE (*).

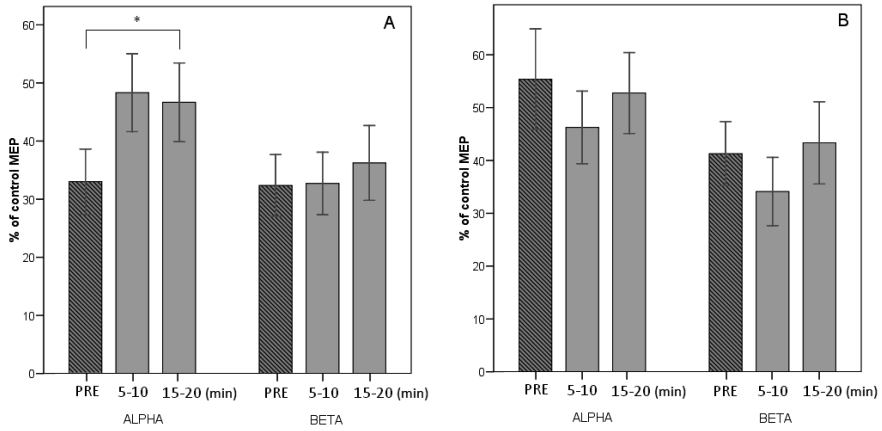

Figure 4. Mean un-trained hemisphere corticospinal excitability (CSE; A) and short intracortical inhibition (SICI; B) 5-10 min (or T_1) and 15-20 min (or T_2) after the ALPHA or BETA protocol. In figure B, higher MEP values indicate disinhibition (reduced SICI). Error bars represent SEM. No significant changes were detected.

Neurofeedback and resting EEG changes are linearly proportional to changes in corticospinal excitability

As depicted for the trained hemisphere in Figure 5A, a scatter plot of alpha training coefficient versus single-pulse MEP amplitude at T_2 for the ALPHA group revealed a significant negative correlation ($r = -0.59$, $p = 0.044$), indicating that the larger the relative decrease in alpha from baseline the greater the increase in corticospinal excitability. Moreover, a parallel *positive* correlation was observed between high gamma mean frequency (60-120 Hz) training coefficient and MEP at T_2 ($r = 0.62$, $p = 0.031$). For the BETA protocol (Figure 5B), the correlation between low beta training coefficient and direction of MEP change was negative at T_1, albeit less robust ($r = -0.53$, $p = 0.08$; Weighted Least Squares regression (WLS) $r = -0.62$, $p = 0.03$). This relationship was negligible at T_2 ($r = -0.25$, n.s.)

When EEG amplitudes were normalised as a percentage of their 3-min baseline value (% T_0), mainly negative correlations occurred between period alpha amplitude and MEP at T_2 (Figure 6), with a trend for increasing significance from the beginning of the session that reached a maximum around periods 6 and 7 ($r < -0.6$, $p < 0.05$), or during 15-21 minutes of NFB.

The resting EEG amplitude change, or ratio of the post-NFB baseline and the pre-NFB baseline power, proved to be another successful predictor of MEP change in all EEG bands below *high* beta ($r < -0.6$, greatest for alpha: $r = -0.71$, $p = 0.01$), suggesting that the more suppressed the slower EEG amplitudes were after NFB, the greater the enhancement of the MEP ~20 minutes later. Moreover, alpha during training periods 7, 8, 9 ($r > 0.6$, $p < 0.05$), but not 10, predicted resting alpha change ($r = 0.65$, $p = 0.02$). As seen in Figure 7 the overall implication is that a 3-way significant association was established between normalised amplitudes of training EEG, resting EEG and corticospinal excitability (CSE).

Analogous analyses were performed on the BETA group for relationships between CSE and normalised low beta amplitudes, disclosing a significant association similar to that found with ALPHA between resting low beta and MEP amplitudes at T1 (Weighted Least Squares r = -0.58, p = 0.050) as well as a borderline significant correlation between training low beta (period 6) and MEP (WLS r = -0.52, p = 0.08). Training low beta amplitude (period 6) was in turn tightly correlated with its subsequent resting amplitude (WLS r = 0.67, p = 0.02), mirroring closely but less reliably, the three-way relationship reported for the ALPHA group. No significant associations were observed between MEP and the remaining EEG bands in the BETA group (e.g. resting alpha vs MEP T1 : WLS r = - 0.17, p = 0.60).

In summary, pre-to-post increases in corticospinal excitability were positively (negatively) correlated with both the sustained time-course and relative degree of desynchronisation (synchronisation) of alpha and low beta rhythms.

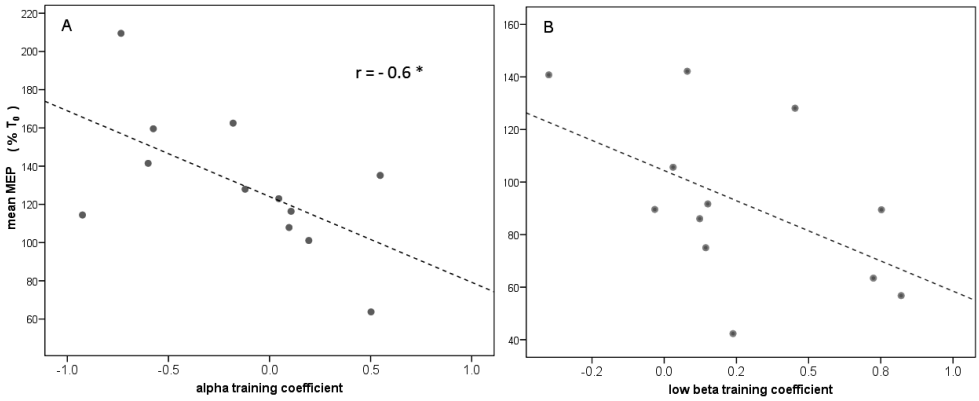

Figure 5. Scatter plots of each participant's (n=12) trained hemisphere NFB training coefficient vs normalised CSE for (A) ALPHA group (r = -0.6) at T_2 and (B) BETA group (r = -0.5) at T_1.

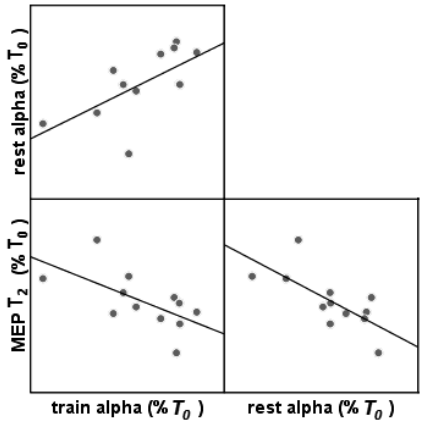

Figure 6. Corticospinal excitability (T_2) vs alpha amplitude correlation, for all ALPHA group trained hemisphere NFB periods. Period number for which the correlation is statistically significant (*).

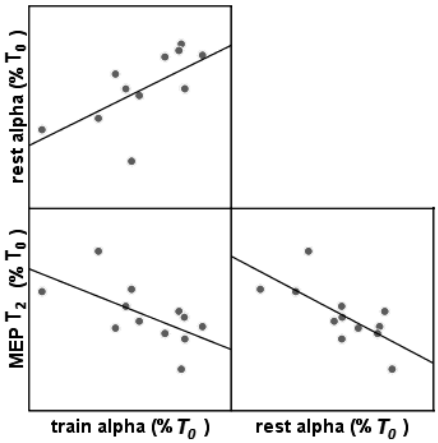

Figure 7. Matrix plot of normalised training alpha (period 7), resting alpha (second baseline), and CSE (T_2) amplitudes in the trained hemisphere. All correlations were significant at $|r| > 0.6$, $p<0.05$.

NFB effects on MEP appear to be indirectly mediated via the resting EEG

To investigate the possible causal relationships between training EEG, resting EEG, and MEP amplitudes, we conducted a path analysis of the three-way correlates linking these variables from our experimental data. For ALPHA group training periods 6, 7, 8, and 9, regression coefficients were consistently higher ($r > 0.5$) for the 2 indirect pathways of training EEG to resting EEG, and resting EEG to MEP, compared to the direct pathway of training EEG to MEP ($r < 0.5$). Figure 8 shows results for ALPHA training during period 7 and MEP at T_2, mirroring Figure 7. Accordingly, a bootstrap test (see Methods for details) revealed a statistically significant ($p < 0.05$) *indirect* effect of training EEG on MEP, *mediated* via the resting EEG change. Moreover, deletion of the train EEG to MEP direct pathway resulted in a better-fit (chi-square = 1.1, df = 1, p = 0.3) and greater parsimony (change in PNFI = 0.31). We then applied this final model to the BETA group relationships described above (low beta amplitude period 6 vs. MEP T_1), which turned out analogous to the ALPHA group, confirming a good-fit mediation model (chi square= 0.4, df = 1, p = 0.5), with the indirect effect having a marginal bootstrap significance of p = 0.08.

Overall, these modelling results suggest that the general NFB effect may be better explained by its action on the resting/spontaneous EEG, which is in turn a more direct modulator of cortical excitability.

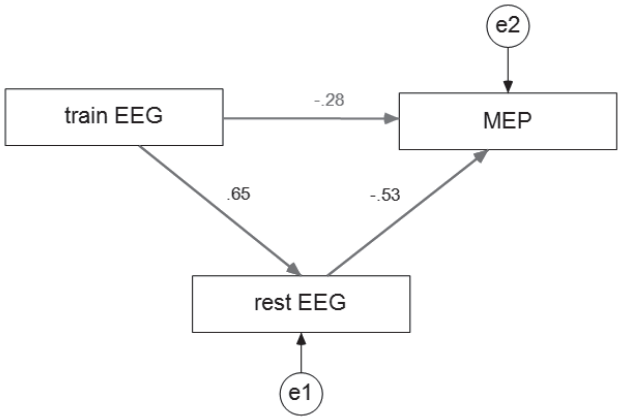

Figure 8. Path diagram of the hypothesized causal relationship between observed training EEG, resting EEG and corticospinal excitability variables. Here, ALPHA group standardised regression coefficients are illustrated for normalised training alpha (period 7), resting alpha (second baseline), and single-pulse MEP (T2) amplitudes in the trained hemisphere. Unobserved residual (error) variables are denoted by e1 and e2.

Intracortical measures are linearly proportional to shifts in the DC potential

Lastly, we explored the association between EEG and the paired-pulse MEP parameters, namely short interval intracortical inhibition (SICI) and facilitation (ICF), which have been found to be coupled to changes in intracortical neuronal circuitry (Lazzaro, Ziemann, & Lemon, 2008). The DC training coefficient was defined as the Pearson correlation between the period number (0 to 10) and the average DC potential (μV) of that period. A positive training coefficient therefore reflects a positive drift in direct current potential during the NFB session. Bearing in mind that increases in SICI amplitude indicate decreases in intracortical inhibition, the ALPHA group demonstrated a negative correlation between the trained hemisphere DC training coefficient and SICI amplitudes at T1 ($r = - 0.6$, $p = 0.04$), T_2 ($r = - 0.53$, $p = 0.07$), and ICF amplitudes at T_2 ($r = -0.79$, $p < 0.01$). Additionally, ICF amplitude at T_2 was positively correlated with SICI amplitude at T_1 ($r = -0.63$, $p = 0.03$) and T_2 ($r = -0.72$, $p < 0.01$). Weaker links were apparent for the BETA group, where borderline negative associations were observed between ICF at T1 and low beta training coefficient ($r = -0.51$, $p = 0.09$) and resting low beta amplitude change ($r = -0.52$, $p = 0.08$).

To conclude, ALPHA group decreases in intracortical inhibition were associated with increases in intracortical facilitation. Moreover, subjects in the ALPHA group who had the most consistent negative shifts in DC potentials displayed the greatest decreases and increases in intracortical inhibition and facilitation, respectively.

Baseline differences

Independent t-tests did not disclose any statistically significant ($p<0.05$) baseline differences between protocol groups for age, measures of EEG band power (delta to high gamma), or TMS measures (resting motor threshold, single-pulse MEP, SICI, and ICF) in either the trained (LH) or untrained (RH) hemispheres.

Discussion

Our findings provide evidence that BCI control of natural human brain rhythms leads to sustained (at least 20 min) changes in motor cortex excitability. They provide support for the view that network oscillations are unlikely to be epiphenomenal and that they may lead to changes in cortical function that outlast their phase of entrainment. Thus, brain oscillations could be an additional mechanism harnessed by the brain to mediate plasticity.

The long-term (>20 min) increase in CSE observed following alpha desynchronisation is unlikely to be a consequence of basic changes in psychological arousal after NFB, since there was a significant correlation between increased amplitude and elapsed time following training, while arousal might have been expected to decrease over the same interval. Arousal also seems an unlikely explanation since low beta (12-15 Hz) training failed to change either CSE or SICI. While we can only speculate as to the mechanisms behind these effects, a slow build up over time is reminiscent of the biochemical cascades known to occur during early long-term potentiation (LTP) [17], as short-term potentiation amplitudes are noticeably extinguished by 15 min [18]. Interestingly for the ALPHA group, MEP increases were negatively correlated with alpha amplitude and positively with high gamma mean frequency. Alpha amplitude reductions have been locally associated with increased motor cortical excitability [14], underlying cortical metabolism [15], attention [19] and globally with behavioral activation [20]. Conversely, alpha synchronisation has been shown to reflect functional inhibition of the motor cortex [12]. On the other hand recent findings have linked high frequency oscillations or high gamma activity with learning [21], attention [2], and increased BOLD activity, neuronal depolarisation and firing rate [22]. In total, this could be a candidate mechanism whereby top-down attention or behavioral activation might prioritise and allocate relevant circuits for neuroplastic change. Moreover, the concomitant reduction in intracortical inhibition (SICI), which is likely to be due to a decrease in cortical GABAergic transmission [11,23] could promote plasticity [24], as previous reports have found an antagonistic effect of GABAergic transmission on motor learning [25] and LTP [26]. The novel finding that SICI was correlated positively, and ICF negatively, with slow shifts in DC potential are compatible with evidence that slow cortical negativities are a marker of increased excitability. However, this was significant for the ALPHA group only and since skin short-circuit was not performed [8], this relationship

awaits replication. Moreover, the apparent lack of correlation of DC measures with the oscillatory EEG is noteworthy, as similar independence has previously been documented for slow cortical potentials and may be suggestive of physiologically separate processes [27]. It also remains unclear whether the release of neuromodulators is a likely mechanism for the overall alpha desynchronisation effects; one attractive candidate may be noradrenalin (NA), which is known to desynchronise alpha rhythms [20], enhance LTP [28], and concomitantly increase CSE and decrease SICI [23].

As low beta entrainment was suboptimal, it is possible that it was associated with an inappropriate training approach in some subjects which was perhaps more desynchronising than synchronising, and therefore counterproductive, hence the slightly increased corticospinal excitability observed later on. This is supported by the negative correlations between low beta training and MEP, which remain in line with findings that low beta synchronisation is associated with motor-cortical deactivation [15] and inhibition [29]. The finding that electrical stimulation of sensorimotor cortex at 10 Hz leads to long term depression (LTD) [30] may be related to the initial inhibitory-like effect observed in this study at a slightly higher, albeit correlated, frequency of 12-15 Hz. Moreover, it has recently been observed that longer durations of 10-Hz repetitive TMS lead to LTD-like effects [31].

It is tempting to compare the average effect size(s) in this study with those of existing noninvasive brain stimulation (NIBS) protocols used to induce neuroplasticity. Repetitive magnetic [32] and direct current [33] stimulation investigations report average corticospinal excitability increases of around 150%, which is comparable to the confidence intervals we observed following alpha desynchronisation. Remarkably, this may indicate that regardless of whether endogenous or exogenous techniques are used, they appear to appeal to a common neural substrate, which is intrinsic to the brain. Crucially however, numerous NIBS protocols induce after-effects that last for periods up to an hour or more. Therefore a question of scientific and therapeutic importance is, how long can the endogenously-driven effects last?

A related issue concerns whether the observed endogenous effects are a direct consequence of longer-term changes to the dynamics of 'resting' or spontaneous rhythms [4,9,14]? This seems to be a tempting account in view of the structural equation model which points to an indirect effect of NFB -*via* the resting EEG- on MEPs. Moreover, this is compatible with online TMS-EEG studies reporting direct modulation of MEPs by cortical oscillations [13,14]. Hence, as EEG rhythms are well-known to be modulated by top-down mechanisms [2,3,34], our observations suggest that the brain may indeed 'shape itself', whereby past activities (as little as ~30 min ago) could in turn determine or bias future states of processing [35]. Here, the concept of a 'background' or stable state would cease to be informative, as such a state would be continually in flux and shaped by present activity. As synaptic homeostasis [36] would need to exert a regulatory role here, a number of studies reporting upregulation of sleep

rhythms after plasticity-induction may further implicate EEG rhythms in synaptic scaling [37,38]. The observation that operant entrainment of 12-15 Hz rhythms enhances spindle rhythms during sleep [16] has recently been replicated, with the finding that it boosts memory recall following sleep [39].

Owing to the noninvasive nature of the experiment, it remains unclear as to exactly where in the brain one could attribute the original cause for the observed effects. One speculation is that thalamocortical circuits could have played a role, as they are known to orchestrate EEG rhythms generated by cortical layer pyramidal cells [4,40]. Hence, the possibility exists that the motor cortex may have been presynaptically modulated by connections from more distributed cortical or subcortical structures. Direct intracellular recordings of corticospinal tract neurons report increased membrane depolarisation during stage shifts towards EEG desynchronisation [41]. In spite of this we did not observe significant changes in the resting motor threshold (RMT), known to reflect variations in membrane conductance [23]. In contrast, two latest studies provide cellular evidence of synaptic changes induced by network oscillations [42,43]. Conversely, changes in synaptic plasticity have been found to modulate neuronal oscillations themselves [42,44]. Our results are moreover compatible with a framework in favour of frequency-dependent forms of synaptic plasticity.

Finally, in recent years a number of investigations have reported behavioural [45] as well as neuronal [46] changes following *long-term* repetitive BCI training. Several neurofeedback protocols [47-50] have been shown to be effective for disorders exhibiting abnormal cortical rhythmicity [51,52]. A latest study induced long-term reductions in resting theta power which were tightly correlated with improvements in clinical attentional-deficit scores [53]. In this respect our results provide a first basis for the 'missing link' between such historical long-term training effects and direct validation of neuroplastic change after an individual session of training. Accordingly, a *repetitive* alpha desynchronisation protocol could be of therapeutic value in pathophysiologies with poor corticomotor activation or increased inhibition; for example, in a disorder such as stroke [54]. It has also been observed that neurofeedback may be useful in facilitating the acquisition of complex sensorimotor skills [55]. Clearly, extensive research is warranted in this method before we can be certain of its neurophysiological mode of action [56]. In light of the extraordinary plasticity displayed by the human brain [57], EEG-based neurofeedback may be a promising technique to modulate cerebral plasticity in a noninvasive, painless, and natural way.

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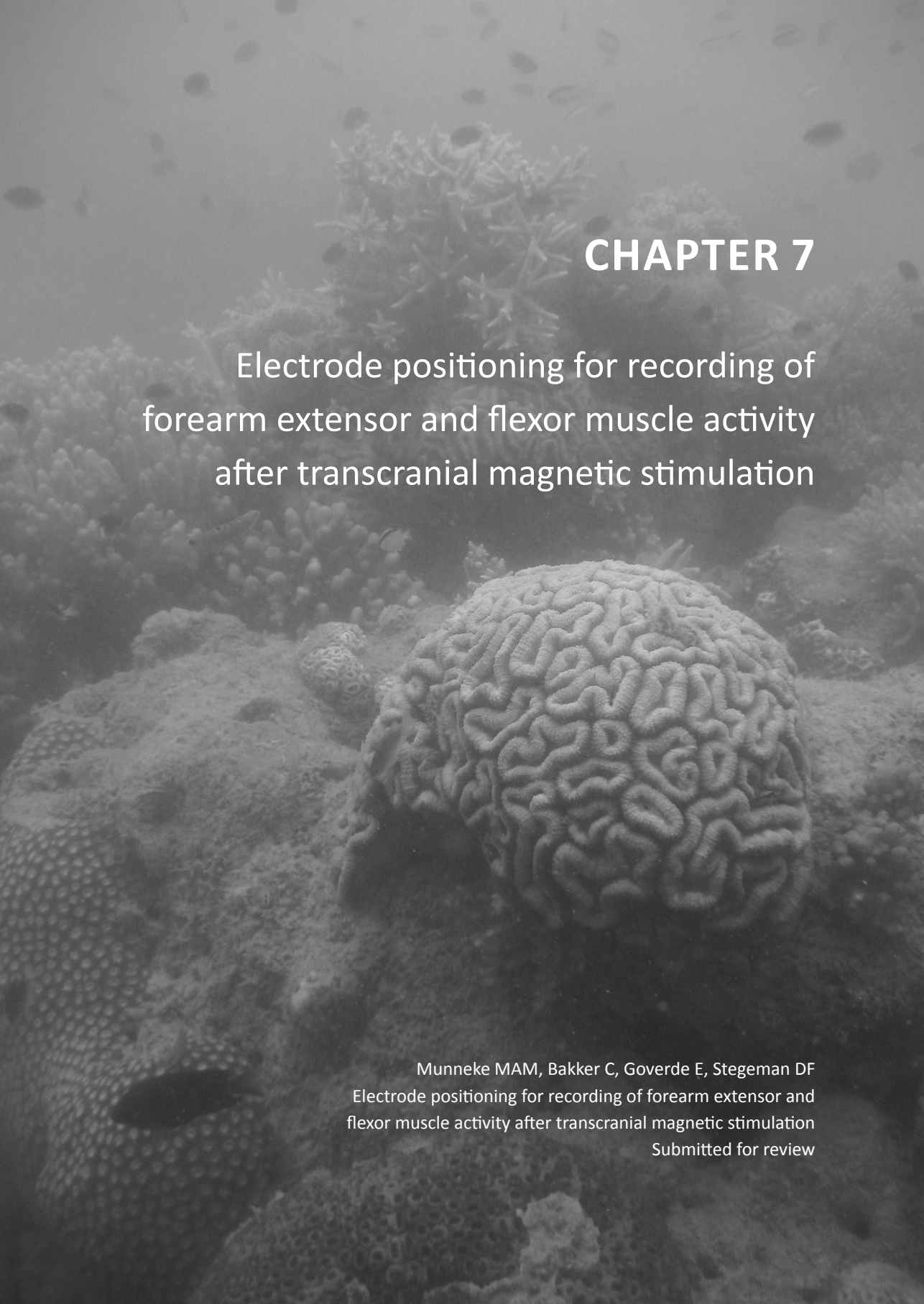

CHAPTER 7

Electrode positioning for recording of forearm extensor and flexor muscle activity after transcranial magnetic stimulation

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Electrode positioning for recording of forearm extensor and flexor muscle activity after transcranial magnetic stimulation
Submitted for review

Abstract

In stroke patients the motor pathways can be affected, leading to paresis. Although it still is hard to reliably predict motor recovery, adding transcranial magnetic stimulation (TMS) has a higher predictive value with respect to motor recovery of the upper extremities than a clinical examination alone. The placement of the surface electromyography (sEMG) electrodes is essential in obtaining information about specific muscle groups and about different corticospinal pathways when using TMS. The goal of this study was to examine which are the optimal sEMG electrode positions for recording muscle activity of forearm flexor and extensor muscles. The first aim concerned optimization of electrode positions to measure the highest motor evoked potential (MEP) amplitudes. The second aim was to find electrode positions that optimally distinguish between these two muscle groups. To be optimally flexible in choosing montages, we used a multichannel sEMG set-up with 37 electrodes around the forearm. The determination of optimal electrode pairs was based on peripheral electrical stimulation. We found pairs that had the highest compound nerve action potential (CMAP) amplitudes and other pairs for the optimal electrode positions to distinguish best between the flexor and extensor forearm muscles. As expected, when applying TMS, flexor and extensor forearm muscles are activated simultaneously. Roughly depending on the interelectrode distances, high amplitude responses or specific muscle group responses can be detected. In conclusion, this study helps to indentify better electrode locations for the use of clinical TMS studies.

Introduction

In routine clinical neurophysiology, transcranial magnetic stimulation (TMS) is used as part of the regular nerve conduction studies. Usually the motor cortex is stimulated in which the magnetically induced current causes activation of the corticospinal motor tracts, resulting in motor responses, so-called motor evoked potentials (MEPs), measured with surface EMG (sEMG) [1]. The placement of the sEMG electrodes is essential in recording signals from specific muscle groups and thus render information about different corticospinal pathways. In stroke patients the motor pathways can be affected, leading to paresis. For the treatment it is important to know which patients have the potential to improve. Although it still is hard to reliably predict motor recovery, adding TMS measurements have a higher predictive value with respect to motor recovery of the upper extremities than a clinical examination alone [2,3]. The objective of this study is to find the optimal sEMG recording electrode positions for the forearm muscle groups. The first aim concerns optimization of electrode positions to record the highest MEP amplitudes. The standard electrode montages described in literature may not be optimal in this context [4].

For the generation of a MEP in a muscle (group) of interest, stimulation has to be applied to the matching region of the motor cortex [5]. A circular TMS coil is most suitable for diagnostic TMS, because of the relatively simple manner of coil positioning and the fact that minor position changes barely influence the results [6]. This implies that with TMS both extensor and flexor muscles will usually be stimulated simultaneously. The second aim of the current study is to find the electrode positions that distinguish best between these two muscle groups. To be optimally flexible in choosing montages, we used a multichannel sEMG set-up with 37 electrodes around the forearm. Straightforward bipolar montages are tested because the clinical setting of the experiment makes it important to test with equipment available on most clinical neurophysiology units.

In order to determine how the compound activity from the extensor and the flexor muscle groups represents itself when these groups are ideally stimulated separately, compound muscle action potentials (CMAPs) after peripheral nerve stimulation were recorded in addition to TMS. The radial and median nerve can be stimulated separately, roughly innervating the extensors and flexors in the forearm independently. On the basis of the less specific character of TMS, it can be expected that the MEP amplitude patterns resemble a superposition of the CMAP patterns from radial and median nerve stimulation. Optimal bipolar electrode positions can be determined for both muscle groups and it was examined whether these electrode pairs were also suitable for TMS.

Methods

Study population

The experiments were performed on 12 healthy volunteers aged 20-24 years (5 men, 7 women). Handedness is associated with asymmetry in the cortical motor representation [7]. Therefore only right-handed subjects participated. Beforehand, the volunteers were screened for health and the presence of metal objects in the body using a standard questionnaire for TMS research [8]. All subjects gave written informed consent prior to inclusion in the study. The ethics committee of the Radboud university medical center approved the study, which was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Electromyography

Multi-channel electromyographic activity was recorded using self-adhesive Ag-AgCl surface electrodes (Kendall Soft-E, H69P; 22x22mm), placed on the forearm. In all subjects, the dominant, right arm was measured. The skin of the forearm was cleansed with alcohol to reduce skin-electrode impedance [9]. Then, the electrode positions were drawn on the forearm. Electrodes were located on the basis of anatomical landmarks for uniformity across subjects [10]. At first, a line was drawn from the olecranon to the apex of the ulnar styloid process (Figure 1). Ring I was placed on 1/3 distance from the olecranon and the second ring approximately 2.4 cm distal to ring I [11,12]. Ring V was located directly proximal to the ulnar styloid process. The location of ring III was halfway between ring I and V, ring IV lay 2.4 cm distal to ring III. At each ring position, the circumference of the arm was measured. Ring I and II consisted of 9 electrodes, each placed with a distance of $1/9^{th}$ of the according circumference. Ring III and IV consisted of 7 electrodes each located on $1/7^{th}$ of the arm circumference and, likewise, ring V counted 5 electrodes placed on $1/5^{th}$ of the circumference. The ground electrode was placed on the lateral epicondyle [13]. After placement of the 37 electrodes, they were attached to a QuickAmp amplifier (72-channel system, Brain Products GmbH, Munich, Germany). EMG signals (average referenced) were recorded, amplified (18.39 nV/bit) and band-pass filtered between 10 and 500 Hz. The EMG signals were acquired at a rate of 2 kHz with the recording software (Brainvision Recorder, Brain Products GmbH, Munich, Germany). The digitized recordings were stored for further analysis.

During the whole session the participants were seated in a chair with the forearm pronated, fully relaxed, and supported by a pillow on the thigh. We used visual EMG feedback to be sure of complete relaxation of the forearm muscles.

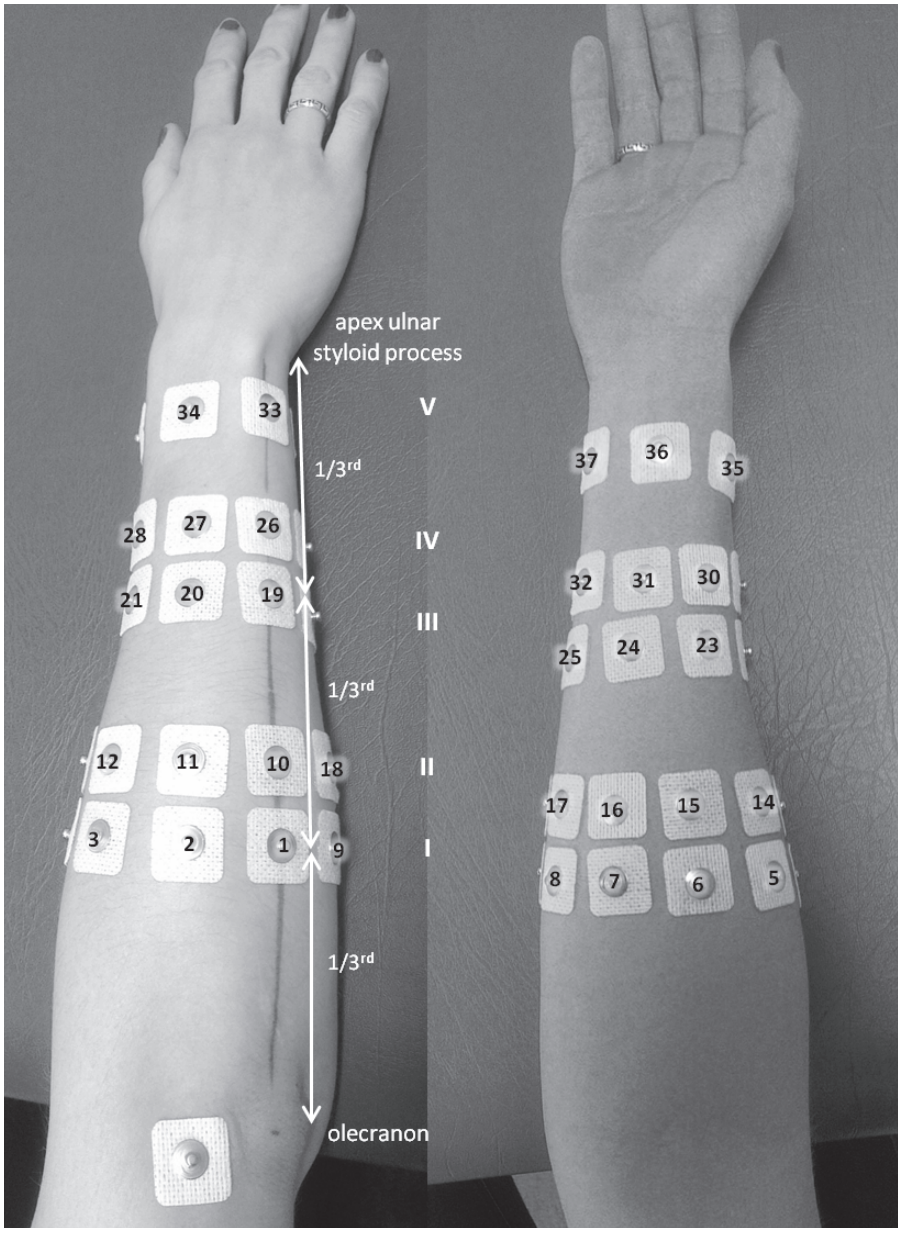

Figure 1. Electrodes were located on the basis of anatomical landmarks for uniformity across subjects. At first, a line was drawn from the olecranon to the apex of the ulnar styloid process. Ring I was placed on 1/3 distance from the olecranon and the second ring approximately 2.4 cm distal to ring I. Ring V was located directly proximal to the ulnar styloid process. The location of ring III was halfway between ring I and V, ring IV lay 2.4 cm distal to ring III. Ring I and II consisted of 9 electrodes, ring III and IV consisted of 7 electrodes and ring V counted 5 electrodes. The ground electrode was placed on the lateral epicondyle.

TMS

The magnetic stimulation was performed using a monophasic Magstim 200[2] magnetic stimulator (Magstim Co., Whitland, Wales, UK) connected to a standard circular coil (remote control 90 mm, Magstim). The coil was centered above the vertex with the A-side visible. Each stimulus induced a counter clockwise current, which resulted in posterior-anterior current flow in the left hemisphere. For the marking of the coil position, a lycra swim cap was placed on the subject's head. The coil was moved lightly in a left laterodorsal direction, making stimulation of the left hemisphere and thus activation of the right arm muscles more optimal [6]. A trigger was sent to the recording software each time a TMS stimulus was given, which facilitated the data analysis.

The TMS stimuli were given with increasing intensity, from 20 up to 100% of the maximal stimulator output (MSO) with steps of 5% and an interval duration of approximately 6 seconds. This method provided 17 different intensities, forming the content of one series. In total 15 series were performed. For the benefit of the subject's and researcher's comfort a break of a few minutes was included after each fifth series.

Peripheral nerve stimulation

After the TMS, electrical stimulation of the median and radial nerve was performed by an experienced clinical neurophysiology technician. A constant current stimulator (model DS7A, Digitimer Ltd, Welwyn Garden City, United Kingdom) was used to produce the electrical stimuli [14]. For the radial nerve, stimulations were given between the biceps brachii and the brachioradialis muscles; for the median nerve between the biceps brachii and the triceps muscles [13]. The trigger intensity was increased until a maximal CMAP was generated. Then, several stimulations were given with that supramaximal intensity. The measurement stopped when three CMAPs were recorded.

Analysis

The analysis was performed using Matlab (MathWorks,Natick, Massachusetts, USA). Concerning TMS, the data of the 15 repetitions of each intensity were averaged per person. For the peripheral stimulation data, an average of the three measured CMAPs was calculated. The averaged MEP and CMAP waveforms were then converted into bipolar data, by means of subtraction of the EMG signal of each electrode from each other electrode (e.g., refer to Figure 1, pair 7-37 consisted of EMG signal from electrode 7 minus the signal from electrode 37). Both for peripheral nerve stimulation and for TMS and for each electrode combination the peak-to-peak amplitude of CMAPs and MEPs was calculated. Per subject and per TMS intensity the CMAP and MEP amplitudes were scaled from 0 to 1 (highest amplitude), this relative amplitude allowed combining and averaging the data over all participants. The amplitude data for each subject, for the group, and for each TMS intensity were analyzed

using a 37 by 37 amplitude map. For the peripheral stimulation two amplitude maps were made per subject, one for the median nerve and one for the radial nerve stimulation. The individual CMAP amplitude maps were checked for consistency between subjects. After that the CMAP maps for the extensor and flexor muscles separately were averaged and for the first aim of this study the electrode pairs with the highest average amplitude were determined. For the second aim of this study, with respect to find electrode positions to distinguish between the flexor and extensor forearm muscle groups, the ratio $CMAP_{median}$/ $CMAP_{radial}$ for each electrode combination was calculated to identify the pair for best isolating the flexor muscle activity and $CMAP_{radial}$/$CMAP_{median}$ (multiplicative inverse of previous ratio) for best isolating extensor muscle activity.
These calculations resulted in four electrode pairs which were used to check the MEP data. The MEP shapes per subject were visually inspected. The relative MEP amplitudes were calculated per subject and electrode combination.

Results

Data

For the calculation of the averaged CMAP amplitude, data of one subject for the median nerve stimulation and data of five subjects for the radial nerve stimulation had to be excluded. Although performed by a skilled technician, in these subjects only adjacent muscles were directly stimulated as was demonstrated by merely isolated EMG activity to the most proximal electrodes close to the stimulation site. For the TMS stimulation, in one subject the stimuli at 95% and 100% MSO were not given because she experienced these as uncomfortable.

Peripheral nerve stimulation

The relative amplitudes of the group CMAP data resulted in two 37x37 amplitude plots: one for the median and one for the radial nerve (Figure 2A and 2B respectively). Clustering of electrode pairs with high amplitudes can be seen. The electrode pairs with the highest amplitude values on group level were selected. For the median nerve active electrode 7 combined with reference electrode 37 gave the highest CMAPs. The individual CMAP amplitude for 7-37 ranged from 0.6 to 1.0, with a mean group amplitude of 0.87. For the radial nerve active electrode 2 combined with reference electrode 35 gave the highest CMAP amplitudes. The individual CMAP amplitude for 2-35 ranged from 0.3 to 0.9, with a mean group amplitude of 0.69.
Figure 2C and 2D show the results of the ratio calculations. Also here, the electrode pairs with the highest values on group level were selected. Electrode combination 7-16 showed to

be the best distinguish the flexor muscle activity ($CMAP_{median}$/$CMAP_{radial)}$. The mean ratio was 9.16 with a range from 2.3 to 16.1. Electrode combination 1-2 showed to the best isolating the extensor muscle activity ($CMAP_{radial}$/$CMAP_{median)}$. The mean ratio was 5.87 with a range from 2.1 to 12.6.

In summary, electrode pairs 7-37 and 2-35 gave highest relative CMAP amplitudes and electrode pairs 7-16 and 1-2 resulted to be the best in distinguishing median from radian innervated muscles.

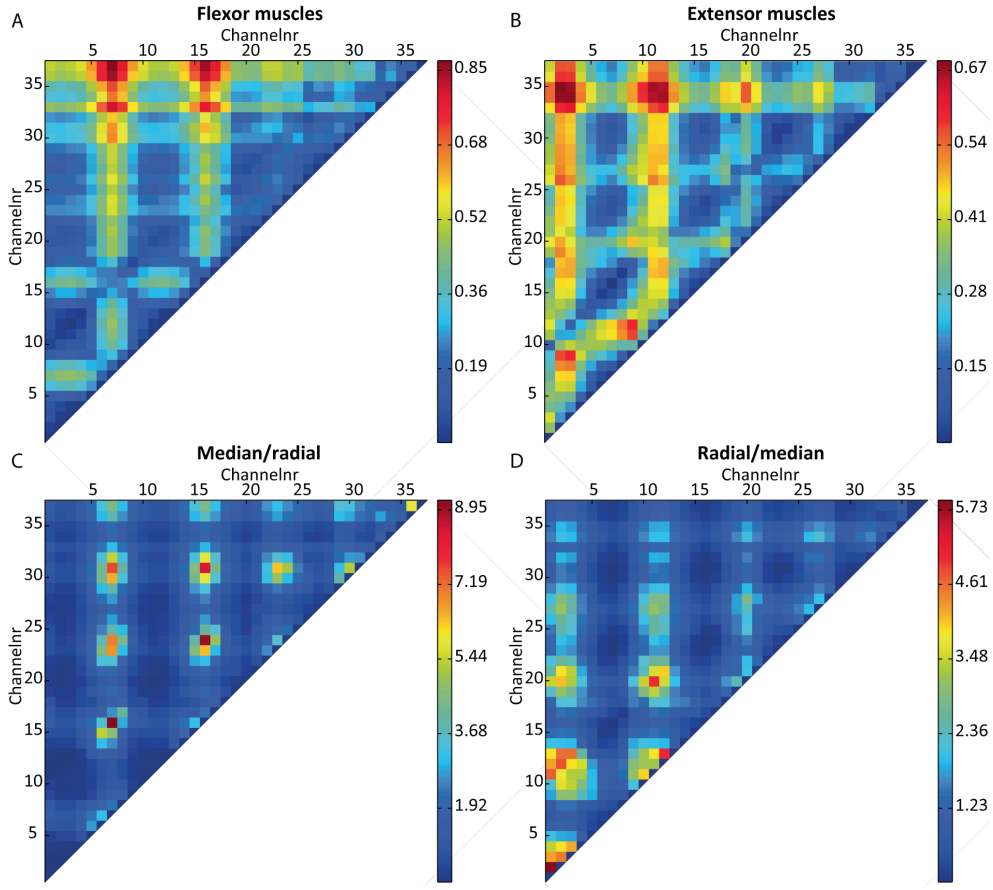

Figure 2. Peripheral nerve stimulation. Relative CMAP amplitude plots for (A) median nerve and (B) radial nerve stimulation for all electrode combinations (37x37). Figure C shows the $CMAP_{median}$/$CMAP_{radial}$ ratio plot and D the $CMAP_{radial}$/$CMAP_{median}$ ratio plot for all electrode combinations.

TMS

For each intensity, a group amplitude plot was produced. The relative amplitude distribution was similar for different stimulation intensities. Therefore, Figure 3 only shows the 37x37 amplitude plot at 90%MSO. As expected a larger amount of electrode pairs showed equal muscle activity, since with TMS it is not possible to only stimulate one forearm muscle group in isolation. Figure 3 can therefore roughly be interpreted as a superposition of the Figures 2A and 2B. The individual relative MEP amplitude for 7-37 ranged from 0.4 to 1.0, with a mean amplitude of 0.69. For 2-35 the mean amplitude was 0.67 (range 0.5 - 0.9). With both electrode pairs substantial MEPs could be measured in all subjects. Figures 4 and 5 show the individual MEP shapes at 90% MSO for the electrode pairs 7-37 (flexor) and 2-35 (extensor), respectively. In most subjects the MEP consists of one negative and one positive peak.

The best electrode pairs for the distinction between the flexor and extensor muscles, as based on CMAP ratios, clearly show lower MEP amplitudes. The individual relative MEP amplitude for 7-16 ranged from 0.1 to 0.4, with a mean amplitude of 0.12. For 1-2 the mean amplitude was 0.19 (range 0.1 - 0.4). This is about a factor five lower than the amplitudes in the electrode combinations 7-37 and 2-35.

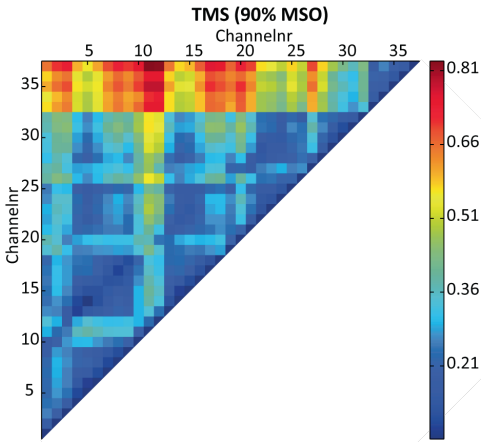

Figure 3. Transcranial magnetic stimulation (TMS). Relative MEP amplitude data at 90% MSO for all electrode combinations (37x37).

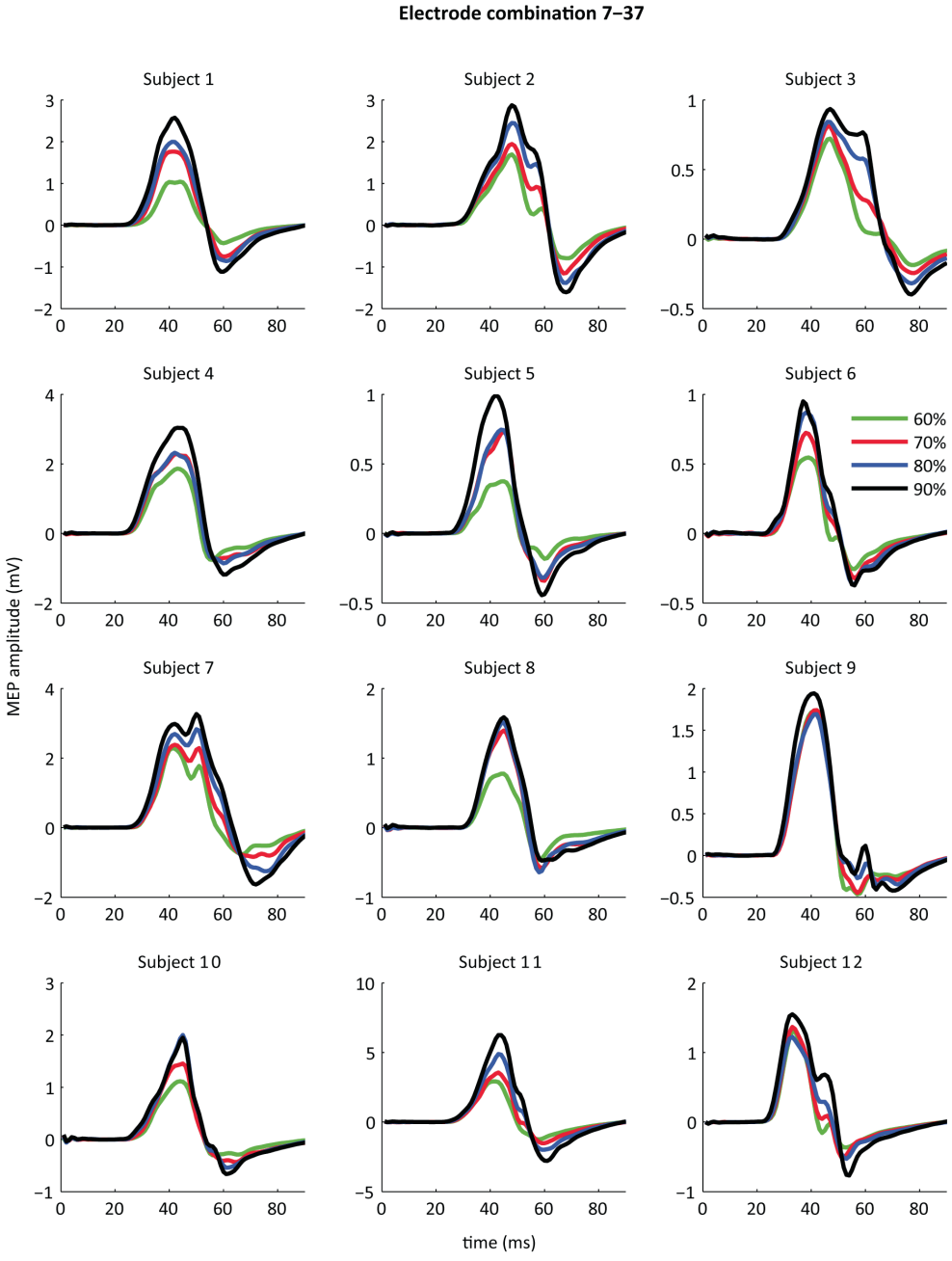

Figure 4. MEP data at 60-90% MSO of electrode pair 7-37, which is marked as the optimal electrode pair to measure a high amplitude response of the flexor muscles.

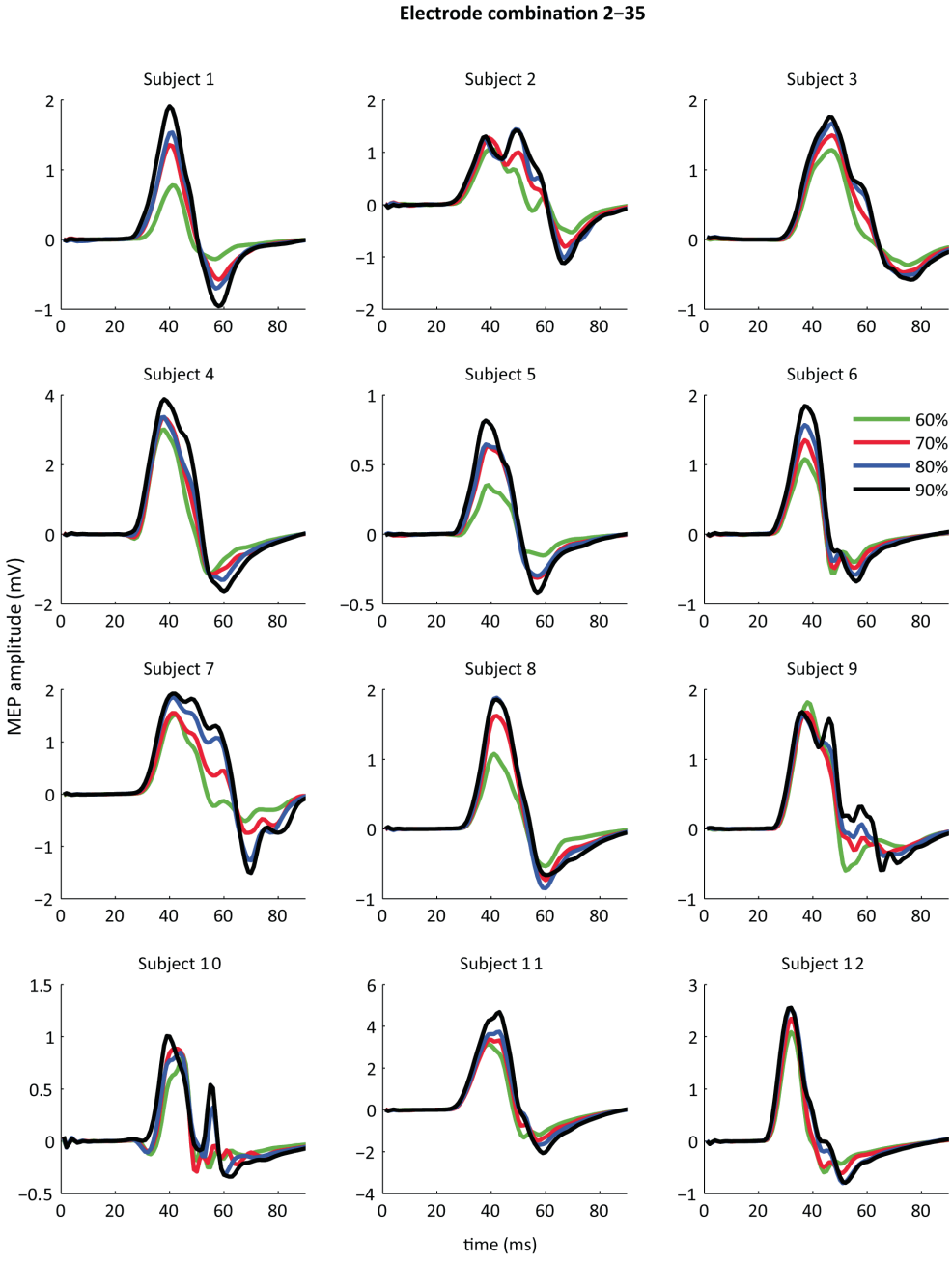

Figure 5. MEP data at 90% MSO of electrode pair 2-35, which is marked as the optimal electrode pair to measure a high amplitude response of the extensor muscles.

Discussion

The goal of this study was to examine which were the optimal sEMG electrode positions for recording muscle activity of forearm flexor and extensor muscles. The first aim concerned optimization of electrode positions to measure for a high motor evoked potential (MEP) amplitude. The second aim of the current study was to find electrode positions to optimally distinguish between these two muscle groups. The determination of optimal electrode pairs was based on peripheral electrical stimulation. We found that the pairs 37-7 and 35-2 (Figure 1A and B) had the highest relative CMAP amplitudes. Pair 7-16 and 1-2 (Figure 1C and D) were the optimal electrode positions to distinguish between the flexor and extensor forearm muscles. After TMS, the amplitude map showed, as expected, less distinct activity of flexor and extensor forearm muscles.

Depending on the aim of a TMS study, the pairs for the highest amplitudes (7-37 and 2-35) and/or for distinction between forearm muscle groups (7-16 and 1-2) can be chosen. In case of TMS measurements after stroke, first goal is to measure the overall integrity by any, even minimal, response of the corticospinal tracts. For such a study electrode pair 7-37 and 2-35 would be advised. If such a response can be detected, the electrode pairs 7-16 and 1-2, although a substantially lower amplitude is predicted, could be used to optimally distinguish activation of different muscles. The latter montages roughly correspond to the SENIAM guidelines: a bipolar muscle specific derivation with two electrodes [4].

The radial nerve is positioned less superficial compared to the median nerve, which made its electrical stimulation harder and led to unintentional direct muscle stimulation. However, the data could still be used because the nerves were nonetheless effectively stimulated. In three subjects, radial nerve stimulation was not possible, leading to exclusion from the analysis. Conclusions based on the remaining data can be considered as still valid. There were no exclusions made regarding the TMS data. In only one subject stimulation was stopped at an intensity of 90% MSO, due to discomfort at the higher intensities. Lack of these data has not hampered the analysis, because results until 90% MSO were sufficient.

A previous feasibility study concluded not to be able to identify optimal electrode pair configurations using conventional sEMG recordings to distinguish between extensor and flexor muscle groups of the forearm in TMS [15]. This conclusion was confirmed in the present study in so far that all electrode distances as used in the previous study are still large and therefore optimal in terms of our first goal. For isolating specific muscle group activity smaller interelectrode distances (IEDs) should used as can be deduced when comparing our Figures 2A,B with Figures 2C,D.

In conclusion, this study contributes to identify better electrode locations for the use of clinical TMS studies. With choosing electrode pairs with large IEDs, information on any minimal sign of nerve tract integrity to the flexor and extensor forearm muscles can be

obtained. To identify MEP activity of a specific muscle (group) shorter IEDs should be used. In the latter case lower or even no observable MEPs in post-stroke patients are the downside. An obvious advice on this basis is to first measure TMS responses using a large IED. In case of sufficient MEP amplitudes, one can repeat the TMS measurements with the above short IEDs for a more specific identification.

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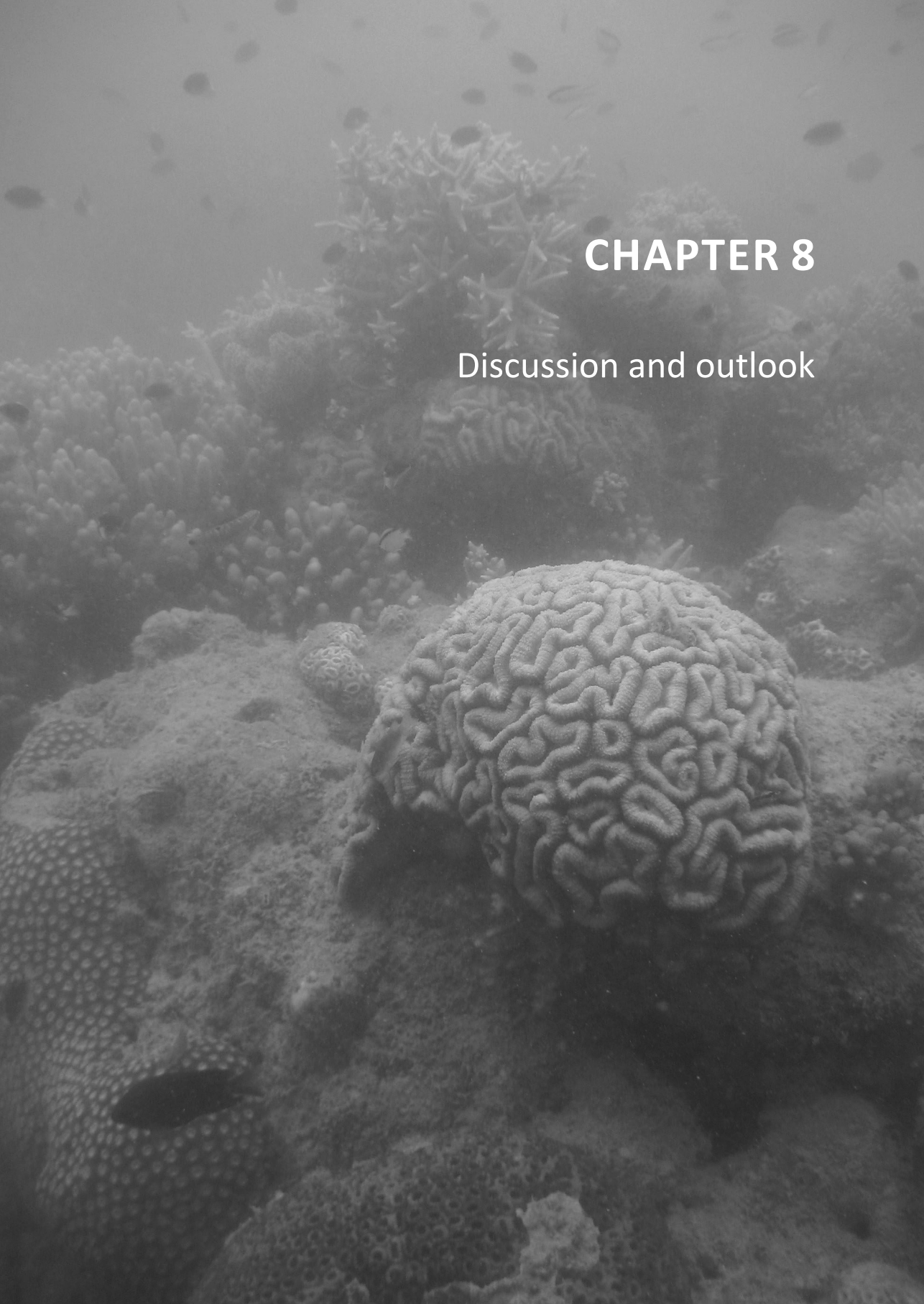

CHAPTER 8

Discussion and outlook

After the introduction by Barker and colleagues of a painless method to excite cortical structures, i.e. stimulation by transcranial magnetic stimulation (TMS), this electrodiagnostic tool very soon became standard equipment in most clinical neurophysiological departments in the later 1980's. With TMS the nerve conduction studies could now also reach the nervous structures of the patient's brain and spinal cord. An example for its use in this context is the assessment of the so-called central motor conduction time in multiple sclerosis, a central nervous demyelinating disease. Nevertheless, the clinical use of TMS never became as popular as was predicted those early days. Its contribution to a (differential) diagnosis appeared limited. In the 1990's, with the introduction of equipment that could deliver repetitive pulses, TMS drew the attention of fundamental neuroscientists. TMS became a method to investigate the brain's state, much more precisely in time than the upcoming brain imaging techniques like functional MRI. With TMS it was possible to interrupt a certain brain process precisely in time, and to modulate the excitability of specific brain structures. The possibility to induce plastic changes for periods extending the stimulation duration, was a welcome addition to the booming brain imaging research.
In the early 2000's, another technique, transcranial direct current stimulation (tDCS), used for animal studies already in the 1960's, re-appeared for human studies. This technique showed the capacity to change cortical function for up to an hour or longer. Both techniques, TMS and tDCS, even have led to studies on their effectiveness in improving the cognitive capacities of healthy subjects, including children.
In the course of time, the clinical interest arose again for more advanced techniques being able to measure and change brain function. Non-invasive brain stimulation became not only a tool in neurological diseases, but also appeared beneficial in psychiatric syndromes like depression. Despite the advanced protocols, the use of "simple" single and double pulse TMS to access cortical excitability, cortical interaction, and the integrity of the corticospinal pathways, remained an essential tool. As a fact, tDCS owed its revival to the availability of single pulse TMS to prove its effect to the primary motor cortex. It was used to quantify corticospinal excitability and interactions. In addition, the effect of self-regulation of cortical activity, for instance by neurofeedback could be quantified by TMS.

The overall goal of the studies in this thesis was the use of non-invasive brain stimulation for measuring and modulating corticospinal excitability and to study the possibility of therapeutic modulation of excitability in a number of neurological disorders. Brain modulation to reduce the over-excitability of the primary motor cortex in ALS is the subject of the Chapter 2 and Chapter 3 in which tDCS and theta burst stimulation (TBS), the latter being as a specific form of repetitive TMS, are used respectively. The effect of TBS over the cerebellum on freezing in patients with Parkinson's disease is described in Chapter 4. Chapter 5 reviews the first studies in literature using TMS as a 'bio'marker in epilepsy

and gives an outlook to its future role. Chapter 6 shows that, in a brain computer interface mediated neurofeedback study, TMS can give "hard proof" on the effectiveness in changing brain excitability. The connectivity between the motor cortex and the muscular system is essential for several neurological disorders like the prediction of recovery after a cerebral vascular accident (CVA). Any muscle response in a paralyzed muscle group after TMS signals the presence of a connection that might be relevant for the chance of functional recovery. A guideline in optimization specifically the recording of electric muscle responses after TMS in the lower arm muscles is the subject of Chapter 7.

Discussion

The results presented in this thesis with respect to modulating corticospinal excitability in ALS (Chapters 2 and 3) are presented here in the recently upcoming awareness that non-invasive brain stimulation protocols have not the consistent effects that were presented in earlier studies. The underlying mechanisms of ''excitatory'' versus ''inhibitory'' aspects of rTMS paradigms should also be taken as relative, because MEP increase after ''excitatory'' rTMS (5-20 Hz) or iTBS might be in fact the result of a decrease of gamma-amminobutyric acid (GABA)-mediated intracortical inhibition (so inhibition of inhibition), rather than a direct enhancement of motor cortex excitability.[1] On the other hand, 1 Hz rTMS and cTBS can enhance the net inhibitory corticospinal control, probably via GABA-B transmission. In fact, it should be considered that the effects of the various TMS protocols suppressing or enhancing cortical excitability are not homogeneous and may result from targeting and modulating various cortical circuits.[2] It has recently been demonstrated that the concept of ''excitatory'' effect of iTBS vs. ''inhibitory'' effect of cTBS on MEP size was highly variable between individuals, depending on differences in the interneuronal cortical networks that are preferentially recruited by the TMS pulse.[3] Another recent study also confirms considerable inter-individual variability in the response of different excitatory non-invasive brain stimulation techniques.[4] Also tDCS must demonstrate comparable effects across a range of people before it can be meaningfully applied in healthy and/or clinical populations. A survey of the literature reveals extensive between- and within-group variation suggestive of an inconsistent effect between individuals.[5] Addressing the inter-individual variability of non-invasive brain stimulation is key to solving issues such as adequate sample sizes in those studies, the poor record of to replication of TMS/tDCS results, and the failure to consistently translate interventions showing promise in pilots studies to clinical practice.

Studying the functional role of the cerebellum in freezing in Parkinsons' Disease with TBS even adds a dimension to the variability aspect. In the brain there is interference between

the different brain structures trough inhibiting or facilitating connections. Chapter 4 we also proposed that facilitating the cerebellar activity might a priori not be caused by a boosting iTBS protocol, but by a protocol like cTBS that inhibits the, themselves inhibiting, Purkinje cells in the superficial cerebellar layer. This could also have led to an increase of freezing following iTBS, but his was not what we found. Continuous TBS had no effect whatsoever and with iTBS freezing was diminished. That even a negative effect did occur for the less affected hand can be seen as a confirmation of the importance and the deviant, probably compensating, role of the cerebellar function at the affected side. A deeper understanding of the mechanisms at hand would need a functional imaging technique like fMRI.

The use of the TMS protocols can also have a more straight forward perspective. In stroke patients the motor pathways can be affected, leading to paresis. For the treatment it is important to know which patients have the potential to improve. In the application of Chapter 7, the occurrence of MEPs in the targeted muscles is tested using TMS. Although it still is hard to reliably predict motor recovery, adding TMS measurements have a higher predictive value with respect to motor recovery of the upper extremities than a clinical examination alone.[6,7]

The level of cortical excitability in each subject at baseline, before the stimulation, is an important source of inter- and intraindividual variability of rTMS effects.[8]. This could explain why rTMS effects on intracortical inhibition depend more on baseline individual values than on stimulation frequency. Generally speaking, previous neuronal activity modulates the capacity for subsequent plastic changes and this major influence refers to processes of homeostatic plasticity and metaplasticity.[9] Therefore, the impact of disease-related plasticity and ongoing pharmacological treatments should also be taken into account when viewing the large variability of biological or clinical effects produced by apparently identical non-invasive brain stimulation protocols.

From therapeutic and rehabilitative perspectives, the main interest of non-invasive brain stimulation resides in the persistence of clinical changes well beyond the time of stimulation. The duration of such after-effects increases with the number of stimuli delivered, and may persist minutes to hours or even days after the end of a session. In this thesis we show that the ALS brain appears to resist the stimulation in short term protocols, but can be “bended” after a ongoing regime of stimulation for several days. Interesting to mention is that the long term effect of repeated TBS was not found in young healthy controls (in preparation). For a long term effect one must be also aware of possible placebo effects in the case of prolonged therapeutic response, with clinical remission persisting up to several months beyond the time of stimulation in patients with chronic disorders.

Last but not least, one must be aware of the safety aspects of the used brain stimulation techniques. Over the last years several safety guidelines were published and recommendations on e.g. the duration of stimulation were provided. [10-12] One of the common side effects is a transient headache that responded to simple analgesics (no migraine characteristics) which in some cases could also be caused by a strenuous study set-up instead of the stimulation itself. The other most common side effect was a nonspecific feeling of discomfort (or weakness). The most feared side effect is the occurrence of seizures during and/or subsequent to a session of non-invasive brain stimulation. In epilepsy patients, this fear is even stronger. Luckily, a seizure is a rare event, associated with a crude risk of 1.4% (4 occurrences in 280 reported epilepsy patients) as reported in the study of Bae and colleagues.[13] In all studies performed for this thesis and all other studies in which the author contributed, no serious side effect occurred.

Outlook

This thesis describes a variety of clinically oriented non-invasive brain stimulation studies. The potential of non-invasive brain stimulation studies as diagnostic, prognostic and therapeutic tools in the described diseases and other disorders are expected to be studied in more detail on larger groups of patients. Since the effects of the described techniques are still limited in duration, it is questionable whether long term therapeutic effects will be obtained. For this further studies will have to focus on protocols that increase the duration of the modulation. For therapeutic effects in neurological diseases, the use of non-invasive brain stimulation should also be considered and systematically studied as an adjunctive therapy in combination with medication, physical therapy or psychotherapy, with the aim of improving or accelerating the efficacy of the treatment.

A disadvantage of the TMS equipment is it size that makes a home application less feasible. In this sense, tDCS as well as a simple neurofeedback training do have *a priori* a much better potential being used at home. In addition to the clinical application, the use of non-invasive brain stimulation to boost healthy brain function is booming, not in the least because of its large economic and societal potential. The ethical consequences of this aspect of the developments certainly deserve attention.

Technically TMS and tDCS techniques can be further optimized. One rather unknown aspect is the exact flow of the magnetically or electrically induced current and thus of electric field strengths in the brain structures. This is an important reason why non-invasive brain stimulation is largely based on trial-and-error experiments. Many computer modeling studies appeared in recent years to predict these fields which of course cannot be measured directly.[14,15] Such predictions need experimental confirmation by proving that a model based

stimulation location is optimal when it indeed gives a maximal effect. Then, of course, the related problem of the precise mechanisms of the techniques at a cortical level needs clarification. In this sense a lot of fundamental work has to be done before we really will be equipped to meet the challenges of the practical applications of non-invasive brain stimulation.

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Summary

The overall goal of the studies in this thesis was the use of non-invasive brain stimulation for measuring and modulating corticospinal excitability and to study the possibility of therapeutic modulation of excitability in some neurological disorders. Brain modulation to reduce the over-excitability of the primary motor cortex in ALS is the subject of the chapter 2 and chapter 3 in which tDCS and theta burst stimulation (TBS), the latter as a specific form of repetitive TMS, are used respectively. The effect of TBS over the cerebellum on freezing in patients with Parkinson's disease is described in chapter 4. Chapter 5 reviews the first studies in literature using TMS as a 'bio'marker in epilepsy. Chapter 6 shows that, in a brain computer interface mediated neurofeedback study, TMS can give "hard proof" on the effectiveness in changing brain excitability. The connectivity between the motor cortex and the muscular system is essential for several neurological disorders like the prediction of recovery after a cerebral vascular accident (CVA). Any muscle response in a paralyzed muscle group after TMS signals the presence of a connection that might be relevant for the change of functional recovery. A guideline in optimization specifically the recording of electric muscle responses after TMS in the lower arm muscles is the subject of chapter 7. In the following part of the present chapter, I will summarize the results of the chapters 2-7 one by one.

Chapter 2

A single session of cathodal tDCS was applied to ten ALS patients and ten aged matched healthy controls. In both groups, the stimulation went well, but we concluded that a single session of cathodal tDCS does not produce an excitability shift in patients with ALS. This is in contrast to the effect in a group of aged matched healthy control subjects, where a single session of tDCS can induce a decrease of cortical excitability. Apparently, the defective cortical structures of ALS patients oppose to the normally effective membrane changes induced by tDCS. The effect in the control subjects and the lack of effect in the ALS patients were also accompanied by a large interindividual variability in the resulting cortical excitability. In patients with ALS this hampers its utility as a diagnostic tool. All in all, our results are not encouraging for the therapeutic effect of tDCS nor its diagnostic potential. However, further studies are warranted, because, to date, only 'one-session-tDCS' has been investigated: repeated cathodal tDCS sessions may provide new insights.

Chapter 3

With this study, we demonstrated that continuous TBS (cTBS), a specific form of repetitive TMS, could safely be applied over 5 consecutive days in ALS patients. Similar to the tDCS results of chapter 2, cTBS was not effective after the first day's session in the patient group, whereas a single session in the healthy aged matched controls was able to reduce the corticospinal excitability. However, our results in ten patients suggest that it is possible to

decrease corticospinal excitability in ALS patients, by means of repeating cTBS over 5 days. In a group of younger volunteers (not presented in the chapter's text), this 5 days protocol did not induce the long lasting decrease of cortical excitability as in the ALS patients. Our results provided also evidence that an interval of 3 weeks between the cTBS sessions as was applied by others in a 1-year follow up study, is too long for the effects to last. However, modulation of corticospinal excitability is possible, and future studies should investigate whether it is possible to obtain a positive effect on disease progression through more continuous forms of cortical modulation.

Chapter 4

We studied the possibility to decrease freezing of the upper limbs by stimulating the cerebellum with two opposite theta burst stimulation protocols. Seventeen Parkinson's disease patients with freezing of gait (FOG) symptoms performed a upper limb task before and after the TBS. Duration and number of freezing episodes in freezing of upper limbs (FOUL) were scored per task in different conditions. The usually boosting intermittent TBS (iTBS) protocol over the cerebellum significantly decreased the freezing duration during the upper limb task in the most affected hand. The inhibiting cTBS protocol did not show an effect on both freezing variables. In conclusion, the findings presented here support the theory about the compensatory mechanism of the cerebellar activity in PD patients without FOUL and FOG and that stimulating the cerebellum in patients with FOG decreases freezing. To clarify the neuronal mechanisms and pathways behind this compensation further research is needed in the form of (f)MRI studies. In addition, more research with modulatory non-invasive brain stimulation is necessary to investigate the function of other superficial brain structures that are involved in PD, such as the supplementary motor area.

Chapter 5

This chapter has given a summary of the literature about TMS of the motor cortex and epilepsy. In both focal and generalized epilepsy, abnormal excitability with decreased intracortical inhibition has been found in the literature. A loss of intracortical inhibition can be seen as a characteristic of the entire epilepsy network. The differences between healthy subjects and patients on the one hand, and between different epilepsy syndromes on the other hand, are too small for diagnostic purposes. Repeated measurements in the same patient may, however, provide useful prognostic information. The predictive value of a change in intracortical inhibition, measured at 250 ms, may be used to provide a more rational introduction of new antiepileptic drugs in patients with epilepsy. Clinical studies should show whether an unchanged inhibition in studies using TMS is predictive of the failure of a drug, so that one does not need to wait for often infrequently occurring subsequent seizures in order to adjust the medication.

Chapter 6

For this chapter, 24 healthy subjects, were randomly allocated to two protocol groups for a single 30-minutes neurofeedback session on alpha rhythm desynchronisation or on low beta rhythm synchronisation in the EEG of one electrode that was placed over the motor cortex. The corticospinal excitability was tested with TMS before and twice after the intervention. Our findings provide evidence that a brain computer interface (BCI) controlling natural human brain rhythms leads to sustained (at least 20 minutes) changes in motor cortex excitability. They support the view that network oscillations are unlikely to be epiphenomenal but that they may lead to changes in cortical function that outlast their phase of entrainment. Thus, brain oscillations could be a mechanism harnessed by the brain to mediate plasticity. The results provide a basis for the 'missing link' between such historical long-term training effects and direct validation of neuroplastic change after an individual session of training. Clearly, additional neurofeedback research on cortical rhytm (de)synchronisation is warranted before we can be certain of its neurophysiological mode of action. In light of the extraordinary plasticity displayed by the human brain, EEG-based neurofeedback may be a promising technique to modulate cerebral plasticity in a non-invasive, painless, and natural way.

Chapter 7

In patients suffering from stroke the motor pathways are often involved, leading to paresis. Although it still is hard to reliably predict motor recovery, adding TMS to other clinical variables that might predict outcome has a better predictive value with respect to motor recovery of the upper extremities than a clinical examination alone. The placement of the surface EMG (sEMG) electrodes is essential in obtaining information about specific muscle groups and about different corticospinal pathways using TMS. The goal of this study was to examine the optimal sEMG electrode positions for recording muscle activity of forearm flexor and extensor muscles. The first aim concerned optimization of electrode positions to measure the highest MEP amplitudes. The second aim was to find electrode positions that optimally distinguish between these two muscle groups. To be optimally flexible in choosing montages, we used a multichannel sEMG set-up with 37 electrodes around the forearm. The study was performed in 12 healthy subjects. The determination of optimal electrode pairs was based on peripheral electrical stimulation. We found pairs that had the highest compound muscle action potential (CMAP) amplitudes and other pairs for the optimal electrode positions to distinguish best between the flexor and extensor forearm muscles. After TMS, the amplitude map showed, as expected, less distinct activity of flexor and extensor forearm muscles. By choosing electrode pairs with large interelectrode distances (IEDs), information on any minimal sign of nerve tract integrity to the flexor and extensor forearm muscles can be obtained. To identify MEP activity from a specific muscle (group)

shorter IEDs should be used. In the latter case the relatively low MEPs in post-stroke patients are the downside. An advice is then to first measure TMS responses using a large IED. In case of sufficient MEP amplitudes, the TMS measurements can be repeated with the short IEDs. This study may help to indentify better electrode locations for the use of clinical TMS studies.

Samenvatting

In de afgelopen decennia is het menselijk brein intensief onderzocht. In dit proefschrift worden verschillende studies beschreven over het meten en beïnvloeden van de hersenen met niet-invasieve stimulatie. Dit hoofdstuk introduceert beginselen van stimulatietechnieken, geeft achtergrondinformatie over drie neurologische aandoeningen en sluit af met een samenvatting van dit proefschrift.

Het motor systeem van het menselijk brein

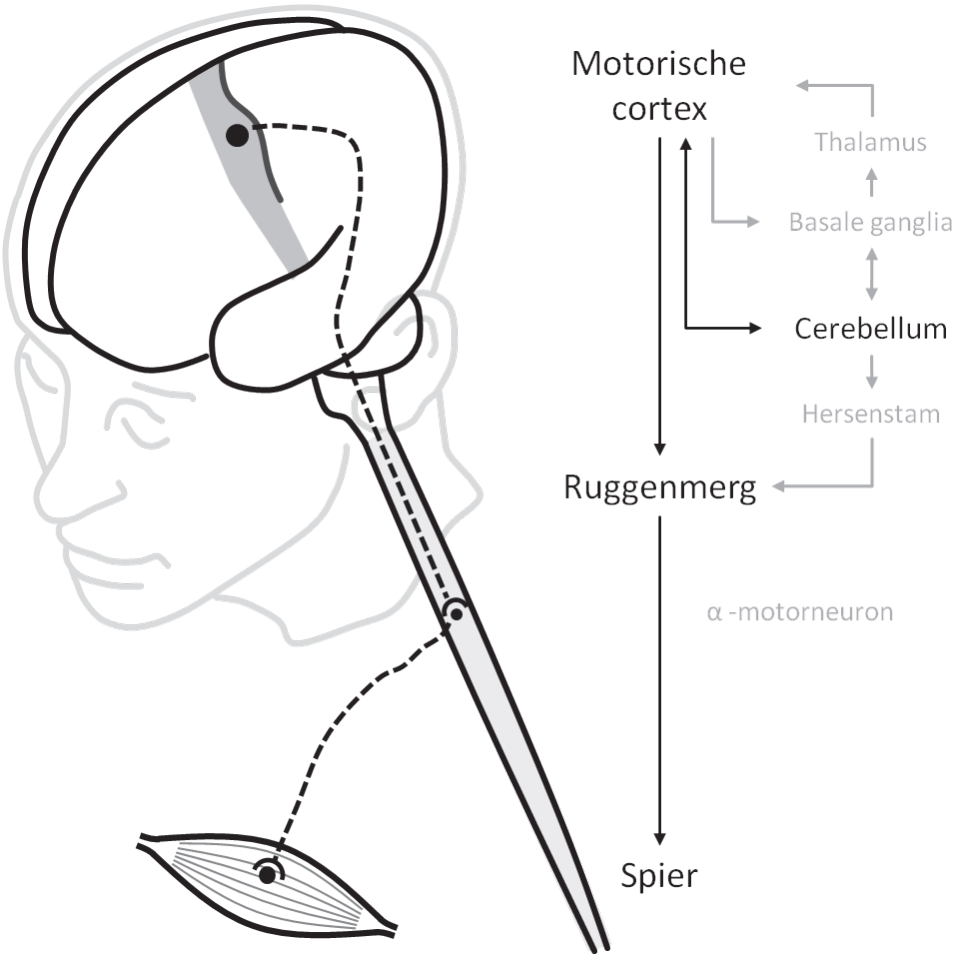
De hersenen is het meest complexe orgaan in het menselijk lichaam. Het is het primaire controle centrum, met miljarden neuronen (zenuwcellen) die gelijktijdig gegevens kunnen verwerken van binnen en buiten het lichaam. Zo zorgt het brein voor de controle van de interne organen, het genereren van gedachten en emoties, het opslaan en oproepen van herinneringen en de controle van beweging. Het belangrijkste deel van de hersenen die verantwoordelijk is voor beweging is de primaire motorische hersenschors (hierna cortex genoemd). Deze cortex ligt op beide hersenhelften en communiceert in een uitgebreid hersennetwerk. De primaire motorische cortex - in de vorm van een lange streep - ligt in de precentrale gyrus vlak voor de centrale sulcus (Figuur 1). Deze primaire motorische cortex activeert spieren of spiergroepen via de corticospinale baan: een route van de hersenen (corticale neuronen) naar het ruggemerg (spinale of αmotorneuronen). Deze activatie gaat rechtstreeks (direct) of via andere belangrijke hersengebieden (indirect): capsula interna, middenhersenen, pons en medulla oblongata, waar 80% van de zenuwvezels oversteken naar de andere (laterale) zijde van het ruggenmerg waar het projecteert op α motorneuronen (spinale neuronen).[1] De α motorneuronen sturen de spieren direct aan door middel van hun functionele bouwstenen, de zogenoemde motor units. De meest directe weg van de primaire motorische cortex naar de spieren is de belangrijkste route voor de spiercontracties opgewekt door TMS (techniek gebruikt in dit proefschrift, zie verderop).

Meten corticale prikkelbaarheid

Geschiedenis

In de 18e en de vroege 19e eeuw worden talrijke studies over menselijke en dierlijke elektriciteit gerapporteerd. Sinds het werk van Galvani en Volta rond 1790, is het bekend dat zenuwen en spieren gestimuleerd kunnen worden door extern opgelegde elektrische stromen. Bij elektrische stimulatie wordt de stroom vervoerd door elektronen in de stoomdraden naar de elektroden en vervolgens bij de elektroden overgebracht als een stroom van ionen in het weefsel. Een klein deel van de lading op deze ionen gaat over op nabijgelegen prikkelbare

membranen en dat kan dan leiden tot membraanontlading. In 1831 ontdekte Michel Faraday het wetenschappelijke principe van de elektromagnetische inductie. Onderzoekers bestudeerden de werking van dit principe op de menselijke hersenen al in de 19e eeuw.[2]



Figuur 1. Schematische en vereenvoudigde voorstelling van het corticospinale systeem met de directe corticospinale route en aanverwante/verbonden hersenstructuren. De primaire motorische cortex is een lange strook cortex gelegen vlak voor de centrale sulcus. De corticale neuronen projecteren direct naar het ruggenmerg en geven directe controle op de α motorneuronen die de spieren activeren.

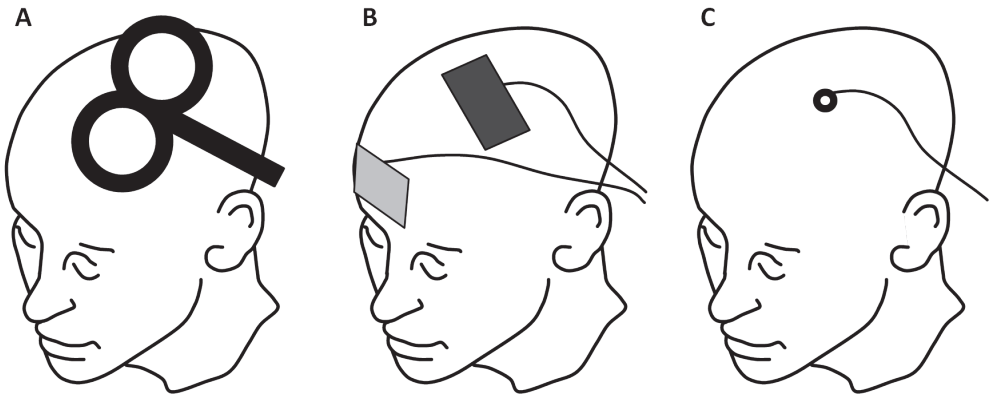
Transcraniële magnetische stimulatie

In 1985 introduceerde Barker en collega's de techniek van transcraniële magnetische stimulatie (TMS).[3] TMS werkt door het passeren van een korte maar grote stroom door een koperen spoel die over de hoofdhuid wordt geplaatst. Door elektromagnetische inductie

veroorzaakt de korte stroom (puls) een groot veranderend magnetisch veld. Vervolgens wekt dat veld een elektrisch veld in de onderliggende hersenstructuren op vanwege de geleidende eigenschappen van het weefsel. Wanneer een enkele puls TMS over de primaire motorische cortex wordt gegeven, stimuleert het elektrische veld direct en/of indirect de corticale motorische neuronen met hun axonen (uitlopers) via de corticospinale baan, naar de in het ruggenmerg gelegen αmotorneuronen. Dit leidt tot een onvrijwillige samentrekking van de spieren aan de andere zijde van het lichaam dan de zijde waar de hersenstimulatie werd gegeven. De elektrische spierreactie wordt een motor evoked potential (MEP) genoemd en kan worden gekwantificeerd met elektromyografie (EMG). De grootte van de amplitude van deze MEP weerspiegelt de prikkelbaarheid van corticospinale system.[4] Het netto effect van TMS is afhankelijk van de positie en oriëntatie van de spoel over een gyrus of sulcus en de richting van de geïnduceerde stroom. Een belangrijk uitgangspunt is dat bij voorkeur axonen - en niet zozeer cellichamen - geactiveerd worden door gepulste neurostimulatie.[5] TMS genereert niet alleen lokale effecten maar ook op afstand via de geactiveerde netwerken.
Naast de zogenaamde enkele puls TMS waren Kujirai en collega's (1993) de eersten die metingen deden naar de corticale inhibitie (remming) en facilitatie (versterking).[6] Zij beschreven een gepaarde puls TMS techniek bestaande uit een conditionerende TMS puls gevolgd door een test puls. Parameters zoals de intensiteit van de pulsen, maar ook de tijd tussen de twee pulsen (interstimulus interval, ISI) bepalen de interacties tussen stimuli. Afhankelijk van het ISI kunnen de inhiberende (SICI) en/of faciliterende (ICF) eigenschappen van samenwerking tussen de neuronen gemeten worden.
In dit proefschrift werden metingen met zowel enkele puls als gepaarde puls TMS uitgevoerd om corticospinale prikkelbaarheid te beoordelen. De hoofdstukken 2, 3, 4 en 6 beschrijven onderzoek met behulp van deze metingen voor en na toepassing van de modulerende hersenstimulatie protocollen zoals hierna besproken wordt.

Modulatie van prikkelbaarheid

In dit proefschrift hebben we drie hersenmodulatie technieken onderzocht: TMS, transcraniële gelijkstroom stimulatie [tDCS] en brain-computer interfacing [BCI] (Figuur 2). Afhankelijk van de stimulatieparameters kan de prikkelbaarheid van de cortex verlaagd of verhoogd worden, welke zelfs na afloop van de stimulatie te meten is. Dit effect biedt de mogelijkheid om corticale reorganisatie te provoceren in de gezonde menselijke hersenen [7], als ook in de door ziekte aangedane hersenen.



Figuur 2. Schematische tekening van de toepassing van transcraniële magnetische stimulatie (TMS, A), transcraniële gelijkstroom stimulatie (tDCS, B) en EEG-registratie als element in een brain-computer interface (BCI, C) over de motorische cortex. De TMS spoel wordt over de motorische cortex geplaatst om de corticospinale prikkelbaarheid te meten en/of moduleren (A). Eén tDCS elektrode wordt over de motorische cortex geplaatst, de andere boven de contralaterale wenkbrauw (B) om de corticospinale prikkelbaarheid te moduleren. Voor de BCI toepassing wordt een EEG-elektrode over de motorische cortex geplaatst en de referentie elektrode over de contralaterale mastoïd (positie op de schedel achter het oor; niet getoond).

Transcraniële gelijkstroom stimulatie

In 1998 werd tDCS opnieuw, na het gebruik ervan in dierproeven in 1960, door Priori en collega's geïntroduceerd.[8] Bij tDCS wordt een zwakke constante elektrische stroom (≥ 1 mA) doorgegeven die gedeeltelijk door de schedel gaat en via de onderliggende structuren de corticale structuren stimuleert. Afhankelijk van de polariteit van de stimulatie vindt er verhoging of verlaging van de corticale prikkelbaarheid plaats. Zogenaamde kathodale tDCS, waarbij de kathode over de primaire motorische cortex en de anode boven de contralaterale wenkbrauw wordt geplaatst, leidt tot verminderde prikkelbaarheid van de motorische cortex in gezonde controles.[9] In tegenstelling tot anodale tDCS (waarbij de elektrodes zijn verwisseld van plek) wat leidt tot een verhoogde corticospinale prikkelbaarheid. Kathodale tDCS werd gebruikt in hoofdstuk 2 van dit proefschrift.

Repetitieve transcraniële magnetische stimulatie

Naast het meten van corticospinale prikkelbaarheid kan TMS ook gebruikt worden voor het veranderen van de prikkelbaarheid van de hersenen. Hiervoor moeten de TMS pulsen snel opvolgend worden toegepast (repetitief, rTMS). Klassieke rTMS bestaat uit een trein van TMS pulsen met een constante frequentie (aantal pulsen per seconde). Afhankelijk van die frequentie neemt de prikkelbaarheid af (~ 1 Hz) of toe (5-20 Hz). De duur van de stimulatie bepaalt de duur van het effect. In 2005 werd het zogenaamde theta burst stimulatie (TBS) protocol geïntroduceerd.[10] "Theta" verwijst naar de 5 Hz frequentie waarmee stimuli

gegeven worden. Dit TBS protocol is kort (≤ 200 s) en resulteert in een langer aanhoudend effect bij gezonde proefpersonen (maximaal 1 uur), dan de klassieke rTMS. Met het oog op een mogelijke therapeutische werking, werd het TBS protocol gekozen in de in dit proefschrift beschreven studies (hoofdstukken 3 en 4).

Brain-computer interface

Dit is een andere niet-invasieve techniek om hersenfunctie te moduleren met een geheel ander karakter. We bestudeerden een zogenaamde brain-computer interface (BCI) om hersengolven van binnenuit tijdelijk te veranderen.[11] Bij een BCI wordt de hersenactiviteit gemeten, gedigitaliseerd en gecodeerd met behulp van een computer en dan teruggekoppeld naar de gebruiker. Door deze acties uit te voeren in een gesloten "neurofeedback" lus (NFB) kan er van binnenuit controle uitgeoefend worden op de natuurlijke werking van de hersenengolven via de corticale netwerken.[12]

Prikkelbaarheid in ziekte

Met de bovengenoemde technieken is het mogelijk verschillen in prikkelbaarheid als gevolg van ziekte te meten en moduleren. Eerder onderzoek toont dat deze technieken veilig zijn voor gebruik bij patiënten. In dit proefschrift is niet-invasieve hersenstimulatie besproken in verband met patiënten met neurologische aandoeningen. Nu volgt een korte inleiding op drie van de ziektebeelden in dit proefschrift.

Amyotrofische laterale sclerose

Amyotrofische laterale sclerose (ALS) is een neurodegeneratieve aandoening van de corticale en spinale motorische (α motor) neuronen. Pathologisch wordt de ziekte gekenmerkt door het verlies van motorische neuronen in de motorische cortex, hersenstam en ruggenmerg. Patiënten overlijden door zwakte van de ademhalingsspieren gemiddeld drie jaar na de eerste verschijnselen.[13] ALS manifesteert zich klinisch als toenemende zwakte van vrijwillig aan te sturen spieren.[14] De klinische kenmerken van ALS weerspiegelen het gecombineerde verlies van corticale neuronen in de motorische cortex (aantoonbaar door spasticiteit, verhoogde peesreflexen en pathologische peesreflexen) en motorneuronen in de hersenstam en het ruggenmerg (aantoonbaar door fasciculaties, spierzwakte en spieratrofie). Hoewel de ontstaanswijze van ALS nog onduidelijk is zijn er wel hypothesen over de ziekteprogressie geformuleerd. De zogenaamde corticomotorneuronale (`dying-forward') hypothese stelt dat ALS een primaire aandoening van de motorische cortex is, met een verlies van de lager gelegen motorneuronen als secundair proces door overvloedige signalen van de bovengelegen connecties.[15] Tot op heden hebben de TMS studies bijgedragen aan inzicht

in het disfunctioneren van de cortex en corticospinale baan bij patiënten met ALS [16], met corticale overprikkelbaarheid als een vroeg kenmerk van sporadische ALS [17] en voorafgaand aan het ontstaan van familiale ALS.[18]

Ziekte van Parkinson

Ziekte van Parkinson (PD) is een neurodegeneratieve aandoening waar voornamelijk het dopaminerge systeem van de hersenen bij betrokken is. Pathologisch wordt PD gekenmerkt door ernstig verlies van dopamineproducerende neuronen in de substantia nigra (een deel van de basale ganglia (figuur 1)). Dit verlies veroorzaakt verschillende bewegingsstoornissen, die toenemen in ernst gedurende de progressie van de ziekte. De klassieke motorische symptomen van de ziekte zijn rusttremor, bradykinesie (traagheid van bewegen), stijfheid en houdingsproblemen. Een ander invaliderend motorisch symptoom bij sommige PD patiënten is het bevriezen tijdens het lopen (FOG). Dit verschijnsel uit zich als korte episodes waarbij de patiënt niet in staat is tot het zetten van een stap of alleen extreem korte en snelle stappen kan uitvoeren. FOG veroorzaakt mobiliteitsproblemen en is een van de meest voorkomende oorzaken van vallen bij PD. Het hersenenmechanisme achter het ontstaan van FOG is nog steeds niet helemaal duidelijk en verder onderzoek hiernaar is nodig. Sommige studies geven aan dat verbindingen tussen de frontale cortex en de basale ganglia veranderd/verminderd zijn en dat input van het cerebellum naar de motorische cortex gedeeltelijk compenseert voor deze verstoring.[19]

Epilepsie

Kenmerkend voor epilepsie is de aanleg voor het genereren van aanvallen. Abnormaal verhoogde prikkelbaarheid van de cortex ligt ten grondslag aan deze aandoening.[20] Over het algemeen is de prikkelbaarheid van de cortex zelfs in de afwezigheid van een aanval abnormaal. Tijdens een aanval verspreiden de overmatige signalen zich via een groter netwerk leidend tot disfunctie in een wezenlijk deel van de hersenen, wat de symptomen veroorzaakt. De diagnose en classificatie van epilepsie wordt meestal ondersteund door een elektro-encefalogram (EEG). In het EEG is meestal alleen tijdens een aanval epileptische activiteit te zien: abnormaal hoge pieken (een afgeleide van de hoeveelheid prikkels). Met TMS is het mogelijk de prikkelbaarheid meer direct te meten. Verschillen in prikkelbaarheid zijn aangetoond in verschillende epileptische syndromen, vlak voor en na een aanval en na een slaaptekort. Echter ook in gezonde broers en zussen van patiënten met zowel gegeneraliseerde als partiële epilepsie is verminderde corticale inhibitie gevonden.[21]

Samenvatting

Het doel van de studies beschreven in dit proefschrift was het onderzoeken van het gebruik van niet-invasieve hersenstimulatie voor het meten en het moduleren van corticospinale prikkelbaarheid en het bestuderen van de mogelijkheid van therapeutische modulatie van prikkelbaarheid in een aantal neurologische aandoeningen. Het verminderen van de overprikkelbaarheid van de primaire motorische cortex bij ALS met behulp van tDCS en TBS is het onderwerp van de hoofdstukken 2 en 3. Het effect van TBS over het cerebellum op FOG bij patiënten met de ziekte van Parkinson wordt beschreven in hoofdstuk 4. Hoofdstuk 5 beschrijft de eerste studies in de epilepsie literatuur waarin TMS als biomarker gebruikt wordt. In hoofdstuk 6 wordt met TMS hard bewijs geleverd dat een BCI neurofeedback protocol meetbare veranderingen in de corticale prikkelbaarheid kan bewerkstelligen. De connectiviteit tussen de motorische cortex en het spierstelsel is essentieel voor verschillende neurologische aandoeningen, zoals bij de prognose van herstel na een beroerte. Als de connectiviteit intact is, kan opnieuw aansturing van de spiergroep vanaf de cortex plaatsvinden en herstel optreden. Wanneer met TMS een spierrespons in een verlamde spiergroep kan worden opgewekt, is dat een teken van een intacte verbinding tussen cortex en spier, met veelal een positief verwachting voor functioneel herstel. Een richtlijn voor optimalisatie voor het meten van de elektrische spierrespons na TMS in de onderarmspieren is het onderwerp van hoofdstuk 7. In het volgende deel van dit hoofdstuk zal ik de resultaten van de hoofdstukken 2-7 één voor één uitgebreider samenvatten.

Hoofdstuk 2

In een enkele sessie werd bij tien ALS patiënten en tien gezonde controles kathodale tDCS toegepast. In beide groepen ging de stimulatie goed, maar we kwamen tot de conclusie dat een enkele sessie van de kathodale tDCS geen verandering in corticospinale prikkelbaarheid teweegbrengt in patiënten met ALS. Dit in tegenstelling tot het effect in de groep met gezonde controle personen, bij wie na een enkele sessie van tDCS een daling van prikkelbaarheid te meten was. Het lijkt erop dat de zieke corticale hersenstructuren in ALS patiënten zich verzetten tegen de, normaal effectieve, membraanveranderingen opgewekt door tDCS. Het effect in de controlegroep en het gebrek aan effect bij de ALS patiënten gaat ook gepaard met grote variabiliteit in de prikkelbaarheid tussen afzonderlijke deelnemers. Bij patiënten met ALS belemmert dit de bruikbaarheid van tDCS als diagnostisch hulpmiddel. Al met al zijn onze resultaten niet bemoedigend voor het therapeutisch effect van tDCS noch haar diagnostische mogelijkheden. Er zijn echter verdere studies gerechtvaardigd, aangezien er tot op heden slechts enkele sessie tDCS studies gedaan zijn: herhaalde toepassing van kathodale tDCS zou nieuwe inzichten kunnen verschaffen.

Hoofdstuk 3

Met deze studie hebben we aangetoond dat continue TBS (cTBS) veilig kon worden gebruikt op vijf opeenvolgende dagen bij ALS patiënten. Net als bij de tDCS resultaten van hoofdstuk 2, was cTBS niet effectief na de eerste dag van de sessie in de groep patiënten, terwijl een enkele sessie in de gezonde controles wel de corticospinale prikkelbaarheid kon verminderen. Echter, onze resultaten bij de tien ALS patiënten suggereert dat door middel van het herhalen van cTBS op vijf opeenvolgende dagen het wel mogelijk is de corticospinale prikkelbaarheid te verlagen. In een groep van jongere gezonde controles (niet opgenomen in de tekst van het hoofdstuk) laat dit vijfdaagse protocol niet een langdurige daling van corticale prikkelbaarheid zien. Onze resultaten verschaffen ook aanwijzingen dat een interval van drie weken tussen zulke vijfdaagse cTBS sessies, zoals werd toegepast door anderen in een follow-up studie van een jaar, te lang is om effect te kunnen hebben. Echter, het is goed te weten dat modulatie van corticospinale prikkelbaarheid mogelijk is. Toekomstige studies dienen te onderzoeken of het met herhaalde corticale modulatie mogelijk is om een positief effect op de ziekteprogressie te bewerkstelligen.

Hoofdstuk 4

Wij onderzochten de mogelijkheid om het bevriezen van de bovenste ledematen (FOUL) te verminderen door het cerebellum te stimuleren met twee tegengestelde TBS protocollen (faciliterend en inhiberend). Zeventien PD patiënten met FOG symptomen voerden een handtaak uit vóór en na de TBS. De duur en het aantal episodes van FOUL werden gescoord per taak met verschillende moeilijkheidsgraden. Het doorgaans faciliterende TBS (iTBS) protocol over het cerebellum verminderde significant de duur van de FOUL tijdens de handtaak in de meest aangedane hand. Het inhiberende cTBS protocol had geen effect op beide FOUL variabelen. Kortom, de hier gepresenteerde bevindingen ondersteunen de theorie van het compensatiemechanisme van de cerebellaire activiteit in PD patiënten zonder FOUL en FOG en dat het stimuleren van het cerebellum bij patiënten met FOG het bevriezen vermindert. De neurale mechanismen achter deze compensatie moeten nog verder onderzocht worden met (f)MRI onderzoek. Daarnaast is meer onderzoek met de modulerende niet-invasieve hersenstimulatie noodzakelijk om de functie van andere oppervlakkige hersenstructuren die betrokken zijn bij PD, zoals de supplementaire motor gebieden, te bestuderen.

Hoofdstuk 5

In dit hoofdstuk is een overzicht van de literatuur over TMS van de motorische cortex en epilepsie gegeven. De literatuur beschrijft zowel in partiële als gegeneraliseerde epilepsie abnormale prikkelbaarheid met verminderde intracorticale inhibitie. Dit verlies van intracorticale inihibitie kan gezien worden als een kenmerk van het gehele epilepsie netwerk. De verschillen tussen gezonde personen en patiënten enerzijds en tussen verschillende

epilepsiesyndromen anderzijds zijn te klein voor diagnostische doeleinden. Herhaalde metingen bij dezelfde patiënt kan echter wel bruikbare prognostische informatie opleveren. De voorspellende waarde van een verandering in intracorticale inihibitie, gemeten op 250 ms, kan worden gebruikt om een rationelere keuze te maken voor een nieuw anti-epileptica bij patiënten met epilepsie. Klinische studies zouden moeten aantonen of een onveranderde inhibitie na het starten van nieuwe medicatie voorspellend is voor het falen van dat anti-epilepticum, zodat men niet zolang hoeft te wachten op, vaak maar zelden optredende aanvallen, om de medicatie aan te kunnen passen.

Hoofdstuk 6

Voor dit hoofdstuk werden 24 gezonde proefpersonen willekeurig toegewezen aan één van de twee groepen. Het protocol van de ene groep was een 30-minuten neurofeedback sessie gericht op alfa ritme desynchronisatie. Bij de andere groep was het protocol gericht op synchronisatie van bèta ritme in het EEG. Bij beide groepen werd de EEG elektrode over de motorische cortex geplaatst. De corticospinale prikkelbaarheid werd gemeten met TMS vóór en twee keer na de BCI interventie. Onze bevindingen tonen aan dat door deze BCI interventie, waarmee de natuurlijke hersenengolven gecontroleerd worden, voor langere tijd (minstens 20 minuten) veranderingen optreden in de corticospinale prikkelbaarheid. Dit ondersteunt de opvatting dat de hersengolven in het breinnetwerk kunnen leiden tot veranderingen in de corticale functie, ook na het stoppen van de “training”. Zo zouden we met het veranderen van hersenengolven een mechanisme in handen hebben om de plasticiteit van binnenuit te sturen. De resultaten vormen een basis voor de missende schakel tussen historische lange-termijn trainingseffecten en directe validatie van neuroplastische verandering na een individuele sessie. Het is duidelijk dat extra neurofeedback onderzoek naar corticale ritme (de)synchronisatie nodig is, voordat we er zeker van kunnen zijn wat het neurofysiologische werkingsmechanisme is. In het licht van de buitengewone plasticiteit van de hersenen kan EEG-gebaseerde neurofeedback een veelbelovende techniek zijn om plasticiteit te moduleren op een niet-invasieve, pijnloze en natuurlijke manier.

Hoofdstuk 7

Bij patiënten met een beroerte zijn de motorische zenuwbanen vaak betrokken en dat leidt tot verlamming van de aangedane spieren. Het is nog steeds moeilijk om het motorisch herstel op betrouwbare wijze te voorspellen. Het toevoegen van TMS metingen naast het bestaande klinisch onderzoek, heeft een betere voorspellende waarde met betrekking tot het motorisch herstel van de bovenste extremiteiten dan alleen een klinisch onderzoek. De precieze plaatsing van oppervlakte EMG (surface EMG, sEMG) elektroden is essentieel om de juiste informatie te verkrijgen over specifieke spiergroepen en afwijkeningen in de corticospinale banen gestimuleerd met TMS. Het doel van deze studie was om de optimale

sEMG elektrode posities te vinden voor het meten van spieractiviteit in de spieren voor het buigen en strekken van de pols en vingers, gelegen in de onderarm. Het eerste doel betrof een optimalisatie van de elektrode posities om de hoogste MEP amplitudes te meten. Het tweede doel was om elektrode posities te vinden die optimaal onderscheid konden maken tussen de twee spiergroepen. Om optimaal flexibel de optimale posities te kunnen kiezen hebben we hiervoor een multi-kanaals EMG opstelling gebruikt met 37 elektroden rondom de onderarm. De studie werd uitgevoerd bij twaalf gezonde proefpersonen. De bepaling van de optimale elektrodeparen werd gebaseerd op de perifere elektrische stimulatie. We hebben paren gevonden met de hoogste spieractiepotentiaal (CMAP) en andere paren om het beste onderscheid te maken tussen de buig- en strekspieren van de pols en vingers. Bij de TMS metingen vertoonde de MEP amplitude “kaart”, zoals verwacht, minder afzonderlijke activiteit van buig- en strekspieren. Door elektrodeparen met grotere afstand tussen de elektrodes (IED’s) te kiezen kan informatie over zelfs een minimale respons van de (deels) intacte zenuwbaan aan de spieren in de onderarm verkregen worden. Om MEP activiteit van een bepaalde spier(groep) te identificeren dienen kortere IED’s te worden gebruikt. De laatste methode is moeilijk bruikbaar in patiënten na een beroerte door de spierverlamming zijn de MEP amplitudes relatief laag en daarmee weinig onderscheidend. Een advies is dan om eerst de TMS reacties te meten met behulp van een grote IED. Bij voldoende grote MEP amplitudes kunnen dan de TMS metingen herhaald worden met de korte IED. Dit onderzoek kan bijdragen tot het identificeren van betere elektrode posities voor het gebruik van TMS in klinische studies.

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Biography

Moniek Munneke was born on October 3rd 1984 in Arnhem, The Netherlands. In July 2003, she received her VWO diploma (pre-university education) from the Olympus College in Arnhem. She started her study Biomedical Sciences in September 2003 at the Radboud University in Nijmegen. She obtained her Bachelor of Science diploma in 2006 and continued with the Master Biomedical Sciences with the specialization in Clinical Human Movement Sciences and a minor in Epidemiology. During her studies she performed a major research internship at the department of Neurology, Clinical Neurophysiology unit of the Radboud university medical centre. With this project, her interest in the non-invasive brain stimulation and its potential clinical applications was drawn. As preparation on her PhD research, she finished her Master with a short research internship at the Sobell Department of Motor Neuroscience and Movement Disorders of the Institute of Neurology in London, United Kingdom. Under supervision of prof. John Rothwell she performed two projects with non-invasive brain stimulation as basis. In August 2008, Moniek graduated with the Master of Science degree. In September 2008, she started her PhD research under supervision of prof.dr.ir. Dick Stegeman and dr. Jurgen Schelhaas at the department of Neurology, Clinical Neurophysiology unit of the Radboud university medical centre. The results of her PhD research are described in this thesis. Next to her PhD research, Moniek was the lab coordinator of the Donders Labs for non-invasive brain stimulation from November 2008 till November 2013. Currently, Moniek is working as lecturer at the Medical Faculty of the Radboud university medical centre.

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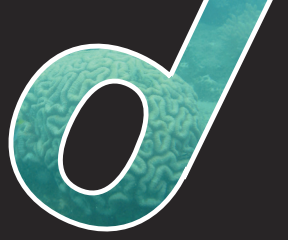

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